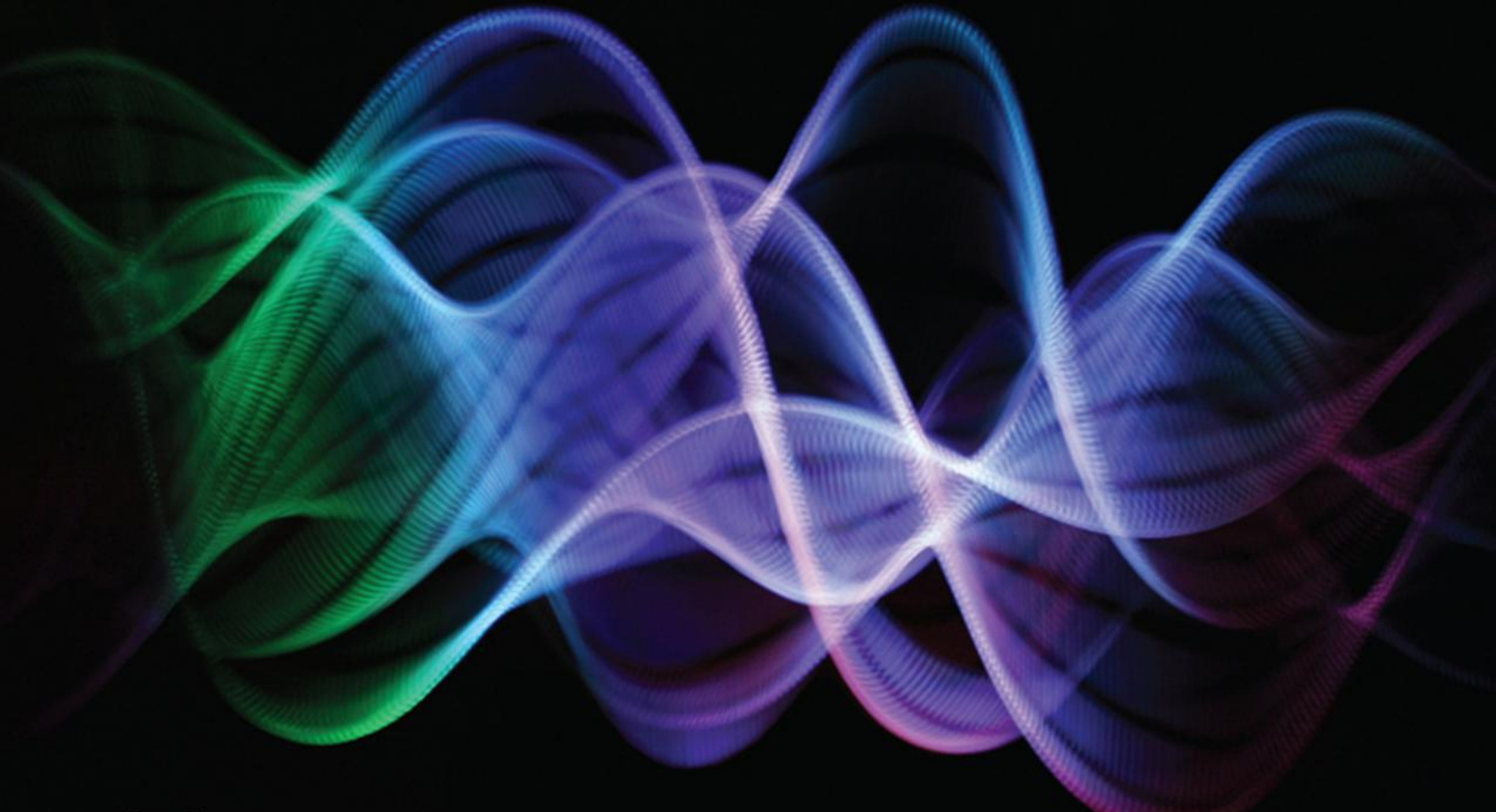
THE CHEMISTRY OF FOOD

JAN VELÍŠEK



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Jan Velíšek
Department of Food Analysis and Nutrition,
Faculty of Food and Biochemical Technology,
Institute of Chemical Technology Prague,
Czech Republic

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Preface

During the 15 years that have elapsed since the first Czech edition of the textbook *The Chemistry of Food* was published, many important monographs and scientific articles from various areas of food science have appeared in the literature, thanks to developments in analytical instrumentation and advances in the knowledge in the field of food technology. All these, plus the interest expressed by the readers, were the impetus that led to the third Czech edition of the book in 2009. This first English edition of *The Chemistry of Food* has remained essentially unchanged in terms of the basic structure of the 12 chapters and the majority of the text, tables, figures and formulae. Certain specific parts, however, have, necessarily, been revised, supplemented and updated.

The Chemistry of Food is the result of many years of experience by workers at the Department of Food Chemistry and Analysis (now Department of Food Analysis and Nutrition), Institute of Chemical Technology in Prague, through the teaching of this subject, plus other related topics taught in the Faculty of Food and Biochemical Technology. The reader will first be introduced to the chemical composition of foods, the important properties of food components and their functions. After these set descriptions, subsequent sections deal with the changes and reactions that occur, or may occur, in foods under certain conditions. It is necessary for the reader to be able to understand the context and complexity of all the processes taking place in foods. As in the previous Czech editions, the authors have tried to reduce to a minimum the amount of text, as is typical for textbooks on biochemistry, microbiology, organic, inorganic and physical chemistry, so that it should not be necessary to consult other specialised textbooks too often, but, at the same time, ensuring the text is clear to both students and professionals.

The textbook contains an introductory chapter and then 11 chapters dealing with the main and accessory nutrients that determine the nutritional and energy value of food raw materials and foods. There is a chapter describing amino acids, peptides and proteins, a chapter dealing with fats, oils and other lipids, as well as chapters on carbohydrates, vitamins, mineral substances and water. Another chapter considers compounds responsible for the aroma, taste and colour attributes that determine the sensory quality of food raw materials and foods. The remaining chapters discuss substances that affect, or may affect, the hygienic–toxicological quality of food raw materials and foods, including antinutritional, toxic and other biologically active food components, food additives and food contaminants.

The third Czech edition featured the work of many workers from the Department of Food Chemistry and Analysis, along with colleagues from other departments of the Faculty of Food and Biochemical Technology and also external authors. Jan Pánek acted as a co-author of the chapters dealing with the main nutrients, and Helena Čížková and Michal Voldřich participated in these and other chapters. Co-authors of the chapter on amino acids, peptides and proteins were Karel Cejpek and Roman Kubec, of the chapter on fats, oils and other lipids Jana Dostálová and Vladimír Filip, of the chapter on carbohydrates Karel Cejpek and Kamila Míková, of the chapter on minerals Richard Koplík, of the chapter on flavour-active substances Jan Šavel, of the chapter on pigments and other colourants Jana Dostálová, of the chapter of antinutritional and toxic substances Pavel Kalač and Přemysl Slanina, and of the chapter on contaminants Jaroslav Dobiáš, Marek Doležal and Jana Hajšlová. The co-authors of the sections dealing with food legislation were Vladimír Kocourek and Kamila Míková. Most of these co-authors were also involved in the first and second Czech editions of this publication. In addition, co-authors of the first and second Czech editions included Jan Pokorný (the chapter on fats, oils and other lipids), Jiří Davídek (the chapter on vitamins and contaminants), Tomáš Davídek (the chapter on carbohydrates), Karel Hrnčiřík (chapters on vitamins and antinutritional and toxic compounds) and Helena Valentová (the chapter on pigments and other colourants). I would like to take this opportunity to thank all of my co-authors and other colleagues for their thorough work. Considerable thanks are also due to the reviewers of the first Czech edition, Prof. Ing. Alexander Príbela, DSc. (Slovak University of Technology in Bratislava, Slovak Republic) and Ass. Prof. RNDr Jan Staněk, PhD, who also did a great deal of hard work on the project.

The co-authors of the Czech editions were Ass. Prof. Ing. Karel Cejpek, Ing. Helena Čížková, PhD, Prof. Ing. Jiří Davídek, DSc., Ass. Prof. Ing. Jaroslav Dobiáš, PhD, Ass. Prof. Ing. Marek Doležal, Prof. Ing. Jana Dostálová, PhD, Prof. Ing. Vladimír Filip, PhD, Prof. Ing. Jana Hajšlová, PhD, Prof. Ing. Vladimír Kocourek, PhD, Prof. Dr Ing. Richard Koplík, Ass. Prof. Ing. Kamila Míková, PhD, Ass. Prof. Ing. Jan Panek, PhD, Prof. Ing. Jan Pokorný, DSc., Ass. Prof. Dr Ing. Kateřina Riddellová and Prof. Ing. Michal

Voldřich, PhD, staff of the Faculty of Food and Biochemical Technology, Institute of Chemical Technology in Prague. External co-authors were Ing. Tomáš Davídek, PhD (Nestlé, Orbe, Switzerland), Ing. Karel Hrnčiřík, PhD (Unilever, Vlaardingen, The Netherlands), Prof. Ing. Pavel Kalač, PhD (University of South Bohemia, České Budějovice), Ing. Roman Kubec, PhD (University of South Bohemia, České Budějovice), Prof. Přemysl Slanina, DVM, PhD (National Food Administration, Uppsala, Sweden), Ass. Prof. Ing. Jan Šavel, PhD (Budweiser Budvar n.p., České Budějovice) and Ing. Helena Valentová, PhD (Hügli Food Ltd, Zásmuky).

Jan Velíšek
Prague, February 2013

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/velisek/foodchemistry

The website includes:

- Powerpoints of all figures from the book for downloading
- PDFs of tables from the book
- Powerpoints of all formulae from the book

1
Introduction

The term **food** means material (eaten or drunk) that contains or consists of nutrients (proteins, fats, carbohydrates, vitamins and minerals) and many other chemical substances. Food is usually of plant or animal origin but, to a lesser extent, it may come from other sources, such as algae or microorganisms. Organisms use food to perform one or more of the four essential functions: to supply energy, to build, repair and maintain body tissues, to supply chemical substances that regulate body processes and to supply chemical substances that protect the organism. Traditionally food was provided through agriculture, but today most of the food consumed by the world's population is either supplied by the food industry or home-grown.

Human food, including such items as meat, poultry, fish, milk, vegetables, fruit and beer for example, is chemically very complex. According to rough estimates, fresh foods contain about half a million different chemical compounds. A much larger number of compounds are the result of biochemical (enzymatic) and chemical (non-enzymatic) reactions that occur during the storage of raw materials and foods and during their industrial and culinary processing.

Food science – the scientific study of food – is one of the major branches of the life sciences. Every aspect of food is covered, integrating and incorporating concepts and information from many different fields, including natural, technical and social sciences. It draws primarily on the knowledge of chemical science (biochemistry, organic, inorganic, physical and analytical chemistry), some areas of physics (such as the mechanics of solids and liquids), biology (especially microbiology), biotechnology, some of the branches of medical science (human nutrition, human physiology, pharmacology, toxicology) and agricultural science (e.g., crop and livestock production, post-harvest plant physiology and *post-mortem* muscle physiology). It also uses a range of experiences from technical engineering disciplines, such as agricultural and food engineering, particularly in the form of genetic food engineering. Food science may also call upon some experience of economics, sociology, psychology and other branches of social science.

The most important building blocks of food science are **food chemistry** and **food technology**. Food chemistry deals not only with the composition of food raw materials and end food products, but also with the behaviour, interactions and reactions of food components and the changes occurring in these food raw materials and end food products under various conditions during production, storage, processing, preparing and cooking. Food technology covers the entire gamut from procuring food raw materials to processing them into food products to preserving, packaging and delivering the food products to the consumer market. The common interest of both disciplines is examination of the possibilities of enhancing the positive changes and preventing the unwanted ones, elimination of antinutritive and toxic food components, and prevention of possible contamination with substances that can pose health risks and guarantee food safety. Producing safe and nutritious food for human and animal consumption is one of the principal aims of both food chemistry and food technology.

The most important natural constituents of foods are the **nutrients** that all organisms need in order to live and grow. The **main** (primary or basic) **nutrients** are **proteins**, **fats** (mainly triacylglycerols and phospholipids, but also numerous other lipids) and **carbohydrates** (such as some poly-, oligo- and monosaccharides) that determine the **nutritional** (nutritive) **value** of food, as they provide structural material (e.g., proteinogenic amino acids used for protein synthesis and lipids from which cell membranes are built) and energy. They may also have other functions. Nutrients are said to be essential if they must be obtained from an external source, either because the organism cannot synthesise them or it cannot produce them in sufficient quantities. Essential main nutrients include not only essential amino acids, but also lipids that are precursors of biologically active substances (such as essential fatty acids and some signalling molecules) and thus have protective functions. Also, some oligosaccharides are biologically active substances (e.g., breast milk oligosaccharides that act as receptor analogues to inhibit the adhesion of pathogens on the epithelial surface).

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The energy value of consumed food depends on the content of nutrients and on such factors as their digestibility, utility, content of some other substances, dietary regime, mental health status and other factors. Energy value is mainly related to proteins, fats and carbohydrates.

Organic nutrients also include **vitamins**. Inorganic chemical compounds, such as dietary **minerals**, **water** (and oxygen), may also be considered nutrients. Vitamins and dietary minerals are collectively known as **accessory (additive) nutrients** and are often referred to as **essential nutritional factors**. With the exception of some vitamins that humans cannot synthesise, these accessory nutrients must be obtained from foods. They are therefore known as **exogenous factors**. Another essential nutrient is water, which is obtained in small amounts by oxidation of primary nutrients, but in much larger quantities from foods, and especially from beverages. Most foods contain a mixture of some or all of the nutrient classes, together with many other substances. Some nutrients can be stored internally (such as the fat soluble vitamins), while others are required more or less continuously. Poor health may be caused by a lack of required nutrients or, in extreme cases, too much of a required nutrient.

Some nutrients, such as proteins, peptides and amino acids, lipids, carbohydrates, vitamins and minerals, are also sensorially active substances (e.g., sweet or bitter). The main nutrients along with other substances (certain organic acids, such as acetic and citric acids, and sugar alcohols, such as glucitol, known as sorbitol) can simultaneously also be a source of energy, for example, ethanol has a relatively high energy value.

In human nutrition, nutrients are often inaccurately classified in a way that reflects the amount that the body requires. These nutrient classes can be categorised as either **macronutrients** (required in relatively large amounts) or **micronutrients** (required in smaller amounts). The macronutrients include proteins, fats, carbohydrates and water; the micronutrients are minerals and vitamins. A third class of dietary material, known as fibre (such as non-digestible polysaccharide cellulose), is also necessary, for both mechanical and biochemical reasons. Other micronutrients include antioxidants and various phytochemicals, which are said to influence or protect some body systems. Their necessity is not well established.

Besides nutrients, foods contain many substances that influence the food sensory impression and its **organoleptic properties**. These food constituents are known as **sensorially active compounds**. They determine the **sensory value** (quality) of foods, inducing an **olfactory** sensation (perception), which is described as the aroma, odour and smell, **gustative** perception, which is the taste, **visual** perception, which is the colour, **haptic (tactile)** perception, which is the touch and feel, and **auditorial** perception, which is the sound. The olfactory sensation is derived from odour-active compounds and the gustative perception from taste-active compounds. Flavour is the sensory impression determined by the chemical senses of both taste and smell and is caused by flavour-active food components. Haptic sensation is the **texture**, which is affected mainly by high molecular weight compounds, such as proteins and polysaccharides, often referred to collectively as hydrocolloids. Geometric aspects of texture that evoke both haptic and visual sensations symbolise the terms **appearance** and **shape**. Aspects of texture related to mechanical properties of food are called **consistency**. Auditorial sensations are associated with a range of textural characteristics (such as crispiness).

Food also contains a range of substances with beneficial or, vice versa, with a negative impact on human health. There is now increasing evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients. Food that does not have a significant history of consumption or is produced by a method that has not previously been used for food is called **novel food**. This was first termed by the European Union (EU) in Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. An example of a novel food is margarine containing phytosterols that help to reduce the blood cholesterol level. Other examples include canola oil produced from rapeseed with low levels of both erucic acid and glucosinolates and exotic fruits and vegetables, which have a long history of safe use. **Functional foods** are defined as foods (beverages) having disease preventing and/or health promoting benefits in addition to their nutritive or processing value. A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in a body, beyond adequate nutritional effects. Functional food can be a natural food, a food to which an existing ingredient has been added, or a food from which a harmful component has been removed by technological or biotechnological processes. The term functional food (officially not used by the EU) was first used in Japan in the 1980s. (FOSHU, Ministry of Health, Labor and Welfare, Japan. Available at: http://www.mhlw.go.jp/english/topics/foodsafety/fhc/02.html.) Examples of functional foods with a long history are table salt fortified with iodine or margarine fortified with vitamin D. Another example is yoghurts with live microorganisms beneficial to the host organism (bacteria of the genus *Bifidobacterium*), which have advantageous effects on health and are called **probiotics**. **Prebiotics** are non-digestible food ingredients (usually oligosaccharides and other substances classified as soluble dietary fibre) that stimulate the growth and/or activity of beneficial bacteria in the small intestine. **Symbiotics** contain both prebiotics and probiotics. **Nutraceuticals** are foods and food products, in some countries physiologically active compounds isolated from foods, which reportedly have a physiological benefit or provide protection against chronic diseases. Such products may range from nutrients (essential amino acids, essential fatty acids, vitamins and minerals), to **dietary supplements** (also known as food supplements or nutritional supplements) and specific diets, to genetically modified foods and other products. Dietary supplements provide nutrients, fibre and various biologically active substances from foods, usually classified according to their effects as antioxidants and anticarcinogens, which may be missing or may not be consumed in sufficient amounts in food. Some countries define dietary supplements as foods, while in others they are defined as drugs or natural health products. Supplements containing vitamins or dietary minerals are included as a category of food in the 'Codex Alimentarius', a collection of internationally recognised standards, codes of practice, guidelines and other recommendations relating to foods, food production and

food safety. **Organic food** is food that is produced using methods that do not involve modern synthetic inputs, such as synthetic pesticides and chemical fertilisers, and does not contain genetically modified organisms or food additives and is not processed using irradiation.

The food components that impair utilisation of nutrients (proteins, lipids, carbohydrates, vitamins and minerals) by biochemical mechanisms are called **antinutritional substances**. An example of such an antinutritional substance is oxalic acid, found in spinach and rhubarb, and which binds up calcium to insoluble calcium oxalate and prevents its absorption. Many foods (especially foods of plant origin) contain various natural toxic substances. These may be toxic only to certain individuals causing an **allergy**, which is related to the immune system response of the organism, or a **food intolerance**, which occurs when food irritates a person's digestive system or when a person is unable to properly digest or breakdown the food. The most common food allergies are peanuts, tree nuts (such as walnuts, pecans and almonds), fish and shellfish, milk, eggs, soy products and wheat. Intolerance to lactose in milk and other dairy products is the most common food intolerance.

Substances toxic to all individuals are called **toxic substances** or **toxins**. Once a toxic substance has contacted the body it may have either acute (immediate) or chronic (long term) effects. Most of food-born toxins are substances with low acute toxicity (such as the pungent alkaloid piperine in black pepper), although some may present chronic effects, such as hepatotoxic pyrrolizidine alkaloids in plants (such as comfrey and coltsfoot species) or cause pathological changes to the respiratory system (e.g., tobacco smoke).

Many chemical compounds in foods (as well as in feeds for livestock) are added intentionally as food **additives**. The purpose is to protect foods against spoilage, oxidation and increase some aspects of the food quality (e.g., nutritional value, aroma, taste, colour or texture). Preservatives, colours and flavours are the best-known additives, but there are in fact many categories of additives, each tailored to a specific purpose.

Contaminants are harmful chemical substances (chemical contamination) and toxic microorganisms (microbiological contamination) that have not been intentionally added to food but may be present there as a result of human activities in agriculture or industry. They may enter food at various stages of its production, packaging, transport or storage. A separate issue is genetically modified food or the presence in food of ingredients from genetically modified organisms, which is sometimes referred to as a form of food contamination. In relation to food, contaminants are sometimes differentiated into primary or exogenous contaminants that come from the external environment (e.g., residues of agrochemicals such as pesticides) and secondary or endogenous contaminants, which are, under certain circumstances, generated during food processing (e.g., by heating and fermentation) from natural food components (nutrients and other constituents). These contaminants are often called technological, processing or process-induced contaminants. They are absent in the raw materials, and are formed by chemical reactions between natural and/or added food constituents during processing. Typical examples are acrylamide formed in potato chips from asparagine, methanol produced in fruit alcoholic beverages by hydrolysis of pectin and chloropropanols formed from fats and hydrochloric acid in hydrolysed vegetable proteins.

Food contaminants and some additives used inappropriately, and sometimes even natural toxic substances, are collectively termed **xenobiotics**. The term is primarily understood to describe artificial substances that do not exist in nature and also covers substances that are present in much higher concentrations than are usual. Their presence in foods is related to the **hygienic–toxicological quality**.

As food chemistry is a dynamic discipline, it also deals with the biochemical and chemical reactions, interactions and physical processes that occur during food processing and storage. Knowledge of these processes is a prerequisite for their eventual regulation and also allows the optimisation of production processes, so that it is possible to produce high value foods in all aspects of quality, foods with high nutritional, sensory and hygienic–toxicological value (healthy foods), while satisfying all the requirements and demands of the consumers. In addition, knowledge of these processes can be applied in ordinary culinary experiences. Finally, food chemistry examines the ways in which to enhance positive changes and to prevent the unwanted ones, eliminate the antinutritive and toxic substances, and prevent possible contamination with substances that can pose health risks to guarantee food safety. Producing safe and nutritious food for human and animal consumption is one of the principal aims of both food chemistry and food technology.

This book takes you on a tour of food chemistry, an incredibly fascinating field of study that will provide you with a constant sense of discovery. For better or for worse, food chemistry is all around us every day and everything in food is chemistry, as chemistry is essential to meet our basic needs of food, energy, health, water and air. Food plays a role in everyone's lives and touches almost every aspect of our existence in some way. Therefore, the magic of food composition and its changes has attracted inquisitive minds for centuries. The understanding of the nature of chemicals and pivotal chemical processes occurring in foods, which the reader will gain, has been divided into 11 chapters, each of which provides insights into different classes of food constituents and related reactions, including proteins, peptides and amino acids, fats and other lipids, saccharides and their derivatives, vitamins, mineral compounds, water, odour- and taste-active substances, colours, beneficial, antinutritive and toxic components, food additives and contaminants and covering all aspects of the subject that are necessary for a comprehensive understanding about how things work. Let us wade into this information soup.

2

Amino Acids, Peptides and Proteins

2.1 Introduction

Aminocarboxylic acids (see p. 5) occurring in nature have vital functions in living organisms. Many of these amino acids are found as free substances or higher molecular weight compounds, where the amino acid building units are connected to each other by amide bonds, –CO–NH–, termed **peptide bonds**. Depending on the size of the molecule (the number of bound amino acids), these compounds are divided into two large main groups, which are:

- **peptides**, usually composed of 2–100 monomers
- **proteins**, which contain more than 100 monomers, but also hundreds or even thousands of amino acids.

Proteins and peptides may even contain some further compounds in addition to amino acids. Proteins are undoubtedly the most important amino acid derivatives: they are the basic chemical components of all living cells and therefore are also part of almost all food raw materials and foods of plant, animal and microbial origin. In organisms, proteins perform a number of unique and extraordinary functions. Along with ribonucleic acids (RNA) and deoxyribonucleic acids (DNA), polysaccharides, some lipids and other macromolecules (such as polyisoprene, the major component of rubber tree latex), peptides and proteins are often known as biological polymers or biopolymers. Nucleic acids (RNA and DNA) have almost no significance as food components in human and animal nutrition, although they play fundamental roles in living systems. Plants and some microorganisms are capable of synthesising proteins from basic substrates such as carbon dioxide, water and inorganic nitrogen compounds, but animals rely on getting their necessary vegetable or animal protein from their food. In the process of digestion, the food proteins are enzymatically broken down (hydrolysed) to their building blocks, from which animals may synthesise their own proteins or use them (along with carbohydrates and lipids) as a source of energy. Therefore, proteins, together with carbohydrates and lipids, belong to the category of **main (primary) nutrients**. In human and animal nutrition, proteins are irreplaceable because they cannot be substituted by other nutrients over a long period of time.

The following sections deal with important amino acids, peptides and proteins, their structure, occurrence, properties, fate in the human organism, nutritional aspects and important interactions and reactions that fundamentally affect the nutritional value, organoleptic properties (odour, taste, colour and texture) and the hygienic–toxicological quality of food commodities.

2.2 Amino acids

Amino acids are found in foods – they are the building units of proteins, peptides and many other compounds – but they also occur as free compounds. Plants, animals and other organisms have been shown to contain more than 700 different amino acids. Some of these are spread quite generally throughout nature, while others occur only in certain species of plants, animals or in other organisms. According to their origins, therefore, the following two groups of amino acids are recognised:

- amino acids found in all living organisms (bound in proteins, peptides or occurring as free amino acids)
- amino acids found only in some organisms (bound in peptides or present as free compounds) that are not protein constituents.

The amino acids bound in proteins (22 compounds) are called proteinogenic, encoded, basic, standard or primary amino acids; 21 of them are constituents of proteins, food raw materials and foods. Of the 22 proteinogenic amino acids, 20 are encoded by the

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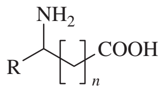
universal genetic code. The remaining two amino acids (selenocysteine and pyrrolysine) are incorporated into proteins by unique synthetic mechanisms. For each of the encoded amino acids, specific transfer RNA molecules exist, and proteins of each organism arise as products of protein synthesis controlled by the genetic code. Amino acids bound in peptides or free amino acids have the same nutritional value as proteins, but their importance in nutrition is usually negligible due to the small amounts in which free amino acids and peptides commonly occur in foods. An example of an amino acid occurring only in some organisms is pyrrolysine, which occurs as a component of enzymes involved in the production of methane in some methanogens, members of a group of single-celled microorganisms of a relatively new domain, Archaea, in the classification of life.

The process of protein biosynthesis is called translation. Post-translational oxidation, alkylation and esterification of some amino acids that are bound in proteins yield modified proteinogenic amino acids (see Section 2.2.1.1.2). Non-proteinogenic (non-encoded, non-standard or secondary) amino acids do not function as building blocks of proteins, as they have other roles in organisms. Amino acids also have an influence on the organoleptic properties of food, especially on their taste. Products of reactions of amino acids are often important compounds influencing odour, taste and colour of foods.

2.2.1 Structure, terminology, classification and occurrence

Amino acids are organic compounds, containing at least one primary amino group, $-NH_2$, together with at least one carboxyl group, –COOH, and various side groups in the molecule. They can be also defined as amino group substituted carboxylic acids. According to the distance of the amino group from the carboxylic group, amino acids (**2-1**) are generally divided into:

- **2-aminocarboxylic acids** (α-aminocarboxylic acids) that have the amino and carboxyl groups attached to the same carbon atom (such as α-alanine);
- **3-aminocarboxylic acids** (β-aminocarboxylic acids) that have the amino and carboxylic groups attached to adjacent carbon atoms (such as β-alanine);
- **4-aminocarboxylic acids** (γ-aminocarboxylic acids) that have one carbon atom (group) between the amino and carboxylic groups (such as γ-aminobutyric acid);
- **5-aminocarboxylic acids** (δ-aminocarboxylic acids) that have two carbon atoms (groups) between the amino and carboxylic groups (such as 5-aminolaevulinic acid, the precursor of the biosynthetic pathway that leads to haem in mammals and chlorophyll in plants);
- **6-aminocarboxylic acids** (ε-aminocarboxylic acids) that have three carbon atoms (groups) between the amino and carboxylic groups (such as lysine).



2-1, 2-amino acid ($n = 0$)
3-amino acid ($n = 1$)
4-amino acid ($n = 2$)
5-amino acid ($n = 3$)
6-amino acid ($n = 4$)

The amino acids also include carboxylic acids that contain a secondary amino group –NH– in the molecule that is a part of three-, four-, five- or six-membered rings. These amino acids are actually derivatives of the saturated nitrogen heterocycles aziridine, azetidine, azolidine (pyrrolidine) and azinane (piperidine) or of other more complex heterocyclic compounds. For example, the only proteinogenic amino acid with a pyrrolidine ring is proline, which in biochemical literature is sometimes incorrectly classified as an imino acid, but imino acids contain imino –C(=NH)– functional groups instead of secondary amino groups.

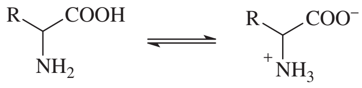
In most foods, about 99% of amino acids are bound in proteins and peptides. The rest (about 1%) are free amino acids. There is a larger amount of free amino acids in foods in which proteolytic enzymes or chemical agents have hydrolysed proteins during manufacture or storage. Larger amounts of free amino acids can be found in some cheeses, beer and wine. The enzymatic hydrolysates of proteins (such as soy sauce) or acidic protein hydrolysates (used as soup condiments) contain only free amino acids with small amounts of peptides, but no protein.

2.2.1.1 Amino acids of proteins

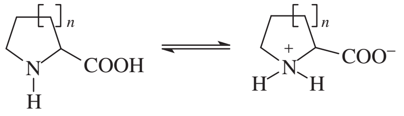
2.2.1.1.1 Proteinogenic amino acids

Proteinogenic amino acids bound in all proteins are exclusively 2-amino (α-amino) acids that have the primary or secondary amino and carboxyl groups attached to the same carbon atom in position 2 (α) to the carboxyl group. In other words, the amino groups are attached to the carbon adjacent to the carboxyl groups. The carbon atom in position 2 (α) is commonly referred to as C_α carbon in biochemical literature.

The proteins in most organisms contain only 21 basic amino acids, of which 20 amino acids have the primary amino group (**2-2**), while one amino acid is an alicyclic amino acid with a secondary amino group (**2-3**). All amino acids except glycine (which is achiral) are optically active (chiral) compounds that contain an asymmetric centre (chiral atom) and thus can occur in two non-superimposable mirror-image forms, D- and L-forms (optical isomers or enantiomers) that are the mirror images of each other. Proteinogenous amino acids almost exclusively belong to the L-series, thus have the L-configuration (see Section 2.2.3.2).



2-2, aliphatic α-amino acid



2-3, alicyclic α-amino acid

One or more hydrogen atoms of the substituent R of L-α-amino acids, known as a side chain, specific to each amino acid (**2-2**), may be substituted by a carboxyl group (called a distal carboxyl group), an amino group (or distal amino group) or other functional groups, such as a hydroxyl group –OH, a sulfhydryl (mercapto) group –SH, a sulfide group –S–CH_3, a guanidino group –NH–C(=NH)–NH_2 or a phenyl group –C_6H_5. The methylene group or nitrogen atom of the secondary amino group of α-amino acids can also be substituted (**2-3**).

The proteinogenic amino acids are often known by their trivial names, which are derived from their properties or the source from which they were first isolated. Systematic names of the proteinogenic amino acids are rarely used, but are more frequent in the non-protein amino acids. Each amino acid has both a three-letter code (mostly the first three letters of the trivial name) and a single-letter code that is used for the registration of long sequences of amino acids in proteins (Table 2.1).

Each classification of amino acids is somewhat imprecise and conforms to certain purposes. The most common way of sorting the proteinogenic amino acids in food chemistry is classification according to the structure of their side chain and functional groups present therein. This will distinguish the following groups of amino acids:

- aliphatic amino acids (monoaminomonocarboxylic acids) with an unsubstituted side chain, which include the simplest amino acid **glycine** (sometimes also classified as the only amino acid without a side chain), its higher homologue **alanine** (known as α-alanine) and branched chain amino acids **valine**, **leucine** and **isoleucine**;

Table 2.1 Trivial and systematic names of proteinogenic amino acids and their codes.

Trivial name	Systematic name	Three-letter code	One-letter code
Glycine	Aminoacetic acid	Gly	G
L-Alanine	L-2-Aminopropionic acid	Ala	A
L-Valine	L-2-Amino-3-methylbutanoic acid	Val	V
L-Leucine[a]	L-2-Amino-4-methylpentanoic acid	Leu	L
L-Isoleucine[a]	L-2-Amino-3-methylpentanoic acid	Ile	I
L-Serine	L-2-Amino-3-hydroxypropanoic acid	Ser	S
L-Threonine	L-2-Amino-3-hydroxybutanoic acid	Thr	T
L-Cysteine	L-2-Amino-3-mercaptopropanoic acid	Cys	C
L-Selenocysteine	L-2-Amino-3-selanylpropanoic acid	Sec	U
L-Methionine	L-2-Amino-4-methylthiobutanoic acid	Met	M
L-Aspartic acid[a]	L-Aminosuccinic acid	Asp	D
L-Glutamic acid[a]	L-2-Aminoglutaric acid	Glu	E
L-Asparagine[a]	L-2-Amino-4-carbamoylbutanoic acid	Asn	N
L-Glutamine[a]	L-2-Amino-5-carbamoylpentanoic acid	Gln	Q
L-Lysine	L-2,6-Aiaminohexanoic acid	Lys	K
L-Pyrrolysine	(2*R*,3*R*)-*N*6-[3-Methyl-3,4-dihydro-2*H*-pyrrole-2-ylcarbonyl]-L-lysine	Pyl	O
L-Arginine	L-2-Amino-5-guanidylpentanoic acid	Arg	R
L-Histidine	L-2-Amino-3-(4-imidazolyl)propionic acid	His	H
L-Phenylalanine	L-2-Amino-3-phenylpropionic acid	Phe	F
L-Tyrosine	L-2-Amino-3-(4-hydroxyphenyl)propionic acid	Tyr	Y
L-Tryptophan	L-2-Amino-3-(3-indolyl)propionic acid	Trp	W
L-Proline	L-Pyrrolidine-2-carboxylic acid	Pro	P

[a]In addition to the specific amino acid codes, three-letter code and one letter-code placeholders are used for ambiguous amino acids, i.e. Asx and B for aspartic acid or asparagine, Glx and Z for glutamic acid and glutamine, Xle and J for leucine or isoleucine and Xaa (Unk) and X for unspecified (unknown) amino acid.

- aliphatic hydroxyamino acids, which include **serine** and **threonine**;
- aliphatic sulfur-containing amino acids **cysteine** and **methionine**;
- selenoanalogue of cysteine called **selenocysteine**;
- amino acids with carboxyl groups in the side chain (monoaminodicarboxylic acids), such as **aspartic acid** and **glutamic acid**;
- their monoamides (amino acids with carboxamide groups in the side chains): **asparagine** and **glutamine**;
- amino acids with basic functional groups in the side chain, that is, diaminomonocarboxylic acid **lysine**, lysine derivative with a 1-pyrroline ring known as **pyrrolysine**, **arginine** with a guanidino group in the side chain and the derivative of 1*H*-isomer of imidazole called **histidine**;
- amino acids with aromatic and heterocyclic side chains, which include **phenylalanine**, its hydroxyl derivative **tyrosine** and **tryptophan** with an indole ring;
- **proline** is the amino acid, in which the functional group is involved in the ring structure.

For clarity, formulae of amino acids are shown in the non-ionised form. Non-ionised molecules are, however, virtually absent in aqueous solutions and in animal and plant tissues. Amino acids are ionised and form inner salts (**2-2** and **2-3**), and carboxyl groups exist as $-COO^-$ anions and amino groups as $-NH_3^+$ cations. The amino acid molecule simultaneously carries positive and negative charges. Because the resulting amino acid contains one positive and one negative charge, it is a neutral molecule called a **zwitterion**. These forms are the predominant ionic forms of amino acids under neutral conditions (about pH 7 in solution). Under these conditions, the distal carboxyl groups of aspartic and glutamic acids, distal amino group of lysine, guanidine group of arginine and imidazole cycle of histidine are also more or less ionised. The nitrogen atoms of the imidazole ring of histidine are denoted by pros ('near', abbreviated π) and tele ('far', abbreviated τ) to show their position relative to the side chain.

In biochemistry, the most common and perhaps most practical classification of proteinogenic amino acids is according to the side chain polarity and its ionic forms occurring in neutral solutions, which is related to non-bonding interactions in proteins (see Section 7.6.2.2). The following groups of amino acids are recognised:

- **hydrophobic amino acids** with non-polar side chains, which include valine, leucine, isoleucine, methionine, phenylalanine, tyrosine and proline; sometimes the hydrophobic amino acids also include glycine, alanine and tryptophan, even though these amino acids are rather amphiphilic amino acids, which forms a transition between the hydrophobic amino acids and the following group;
- **hydrophilic amino acids** with polar side chains, which include serine, threonine, cysteine, selenocysteine, aspartic and glutamic acids and their amides asparagine and glutamine, as well as lysine, pyrrolysine, arginine and histidine.

Hydrophilic amino acids are classified according to the ionic form in which they occur in living organisms into:

- **neutral** (polar side chain has no electric charge in neutral solutions), which includes most amino acids;
- **acidic** (polar side chain has a negative charge in neutral solutions), which includes aspartic acid and glutamic acid;
- **basic** (polar side chain has a positive charge in neutral solutions), such as lysine, pyrrolysine, arginine and histidine.

According to the significance in human nutrition, proteinogenic amino acids are divided into:

- **essential** (or indispensible: valine, leucine, isoleucine, threonine, methionine, lysine, phenylalanine and tryptophan) that cannot be synthesised by the human body;
- **semi-essential** (arginine and histidine);
- **non-essential** (other amino acids; tyrosine forms by hydroxylation of the essential amino acid phenylalanine) that are synthesised by the human body *de novo*.

Amino acids that the body cannot synthesise must be obtained entirely from food. These amino acids are called **essential amino acids**. Essential amino acids are routine constituents of most protein-based foods or dietary proteins and are fairly readily available in a reasonably well-balanced diet. However, there are some amino acids that are present in lower concentrations than the same amino acid in a high quality protein (e.g. lysine in wheat and eggs). These amino acids are called **limiting amino acids**, because if a person's diet is deficient in one of them, this amino acid will limit the usefulness of the others (and will limit the extent of protein synthesis in the body), even if the others are present in what would otherwise be large enough quantities. Limiting amino acids are distinct from non-essential amino acids that the body can synthesise and are therefore sometimes used for food and animal feed enrichment.

In rapidly growing organisms (such as infants, for instance), some non-essential amino acids (arginine and histidine), which the young organism cannot synthesise in sufficient quantities, become essential amino acids. These amino acids are sometimes called **semi-essential amino acids**. Arginine is synthesised at a rate that is insufficient to meet the growth needs of the body, and the majority of it that is synthesised is cleaved to form urea. Histidine was initially thought to only be essential for infants, but longer-term studies established that it is also essential for adult humans. The amino acids methionine and phenylalanine are also sometimes considered semi-essential for reasons not directly related to lack

of synthesis. Methionine is required in large amounts to produce cysteine if the latter amino acid is not adequately supplied in the diet. Similarly, phenylalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet.

The amino acids that the body can synthesise are called **non-essential amino acids**. They are biosynthesised from intermediates of the citric acid cycle used by all aerobic organisms to generate energy and through other metabolic pathways (glycolysis and the pentose cycle). All living organisms, including humans, synthesise glutamic acid from 2-oxoglutaric acid (derived from the citric acid cycle) and ammonium ions. This reaction is known as reductive amination. Glutamic acid then becomes the precursor of glutamine, proline, ornithine and arginine. Transamination of oxaloacetic acid (from the citric acid cycle) yields aspartic acid that is a precursor of asparagine. Transamination of pyruvic acid (a product of glycolysis) yields alanine. Another intermediate of glycolysis, 3-phospho-D-glyceric acid (and D-ribulose 1,5-bisphosphate in plants), yields serine, which is a precursor of glycine, cysteine and selenocysteine. D-Ribose 5-phosphate from the pentose cycle (the product of photosynthesis in plants) is a precursor of histidine.

Essential and semi-essential amino acids

- **Valine** Valine occurs in animal and plant proteins (meat, cereals) in amounts of 5–7% (average content is 6.9%). Egg and milk proteins contain 7–8% valine. The structural protein elastin has the highest amount of valine (16%).

- **Leucine** Leucine occurs in all common proteins, usually in amounts of 7–10% (average content is 7.5%). Cereals contain a variable amount of leucine, wheat proteins contain about 7% and maize proteins about 13%. Free leucine forms in larger amounts during cheese ripening due to bacterial activity.

- **Isoleucine** The largest amounts of isoleucine are found in milk and egg proteins (6–7%), while meat and grains contain 4–5% of leucine (average content is 4.6%).

- **Threonine** A rich source of threonine is meat and brewer's yeast. Its content in animal proteins (meat, eggs and milk) is around 5%. There is a relatively high amount of threonine in wheat, but its content in other cereals is lower (often around 3%), meaning that threonine sometimes becomes the limiting amino acid.

- **Methionine** Animal proteins contain 2–4% of methionine, whereas plant proteins contain only 1–2% (average content is 1.7%). Methionine is the limiting amino acid in legumes. Methionine (and cysteine) is present only in small amounts in histones and is completely lacking in protamines.

- **Lysine** The average content of lysine in proteins is 7%. High amounts of lysine are found in most animal proteins; meat, eggs and milk proteins commonly contain 7–9% of lysine, fish and shellfish proteins contain 10–11% of lysine. In contrast, vegetable proteins, such as proteins of cereals (especially gliadins, but not glutelins) and cereal products contain only 2–4% of lysine, which is the limiting amino acid.

- **Phenylalanine** Good sources of phenylalanine are meat, fish and most protein containing foods, where this amino acid occurs in sufficient amounts (4–5%, average content is 3.5%). In some individuals, its presence in the diet causes phenylketonuria, an inherited defect in which phenylalanine is incompletely and abnormally metabolised (see Section 10.3.1.2).

- **Tryptophan** The average tryptophan content in proteins is 1.1%. Animal proteins contain 1–2% of tryptophan, except for histones and collagen, which do not contain tryptophan at all. Tryptophan is also not present in gelatine or in acid protein hydrolysates used as soup seasonings. Therefore, the tryptophan content in meat products can serve as an indicator of the quality of the meat used. (The contents of 3-methylhistidine or creatinine have been measured for the same purpose.) Cereals contain $<1\%$ of tryptophan; the glutenine fraction of gluten has a somewhat higher content of tryptophan. In fruits and fruit juices N^1-(β-D-glucopyranosyl)-L-tryptophan is also found. Its amount in juices ranges from 0.1 to >10 mg/l. The highest amount of this glycoconjugate was found in pear juices (13.5 mg/l). Humans can partly use tryptophan for biosynthesis of nicotinic acid (see Section 5.8.3). In ruminants, if present in excessive amounts in the diet, tryptophan causes pulmonary oedema (fluid accumulation in the lungs) that may cause respiratory failure. The cause is the heterocyclic compound 3-methylindole (skatole), which is a fermentation product of tryptophan in the rumen.

- **Arginine** Arginine occurs in all proteins in amounts of 3–6% (average content is 4.7%). Basic proteins protamines from fish roes have particularly high levels of arginine. Peanuts and other oilseeds are also rich sources (arginine content of up to 11%).

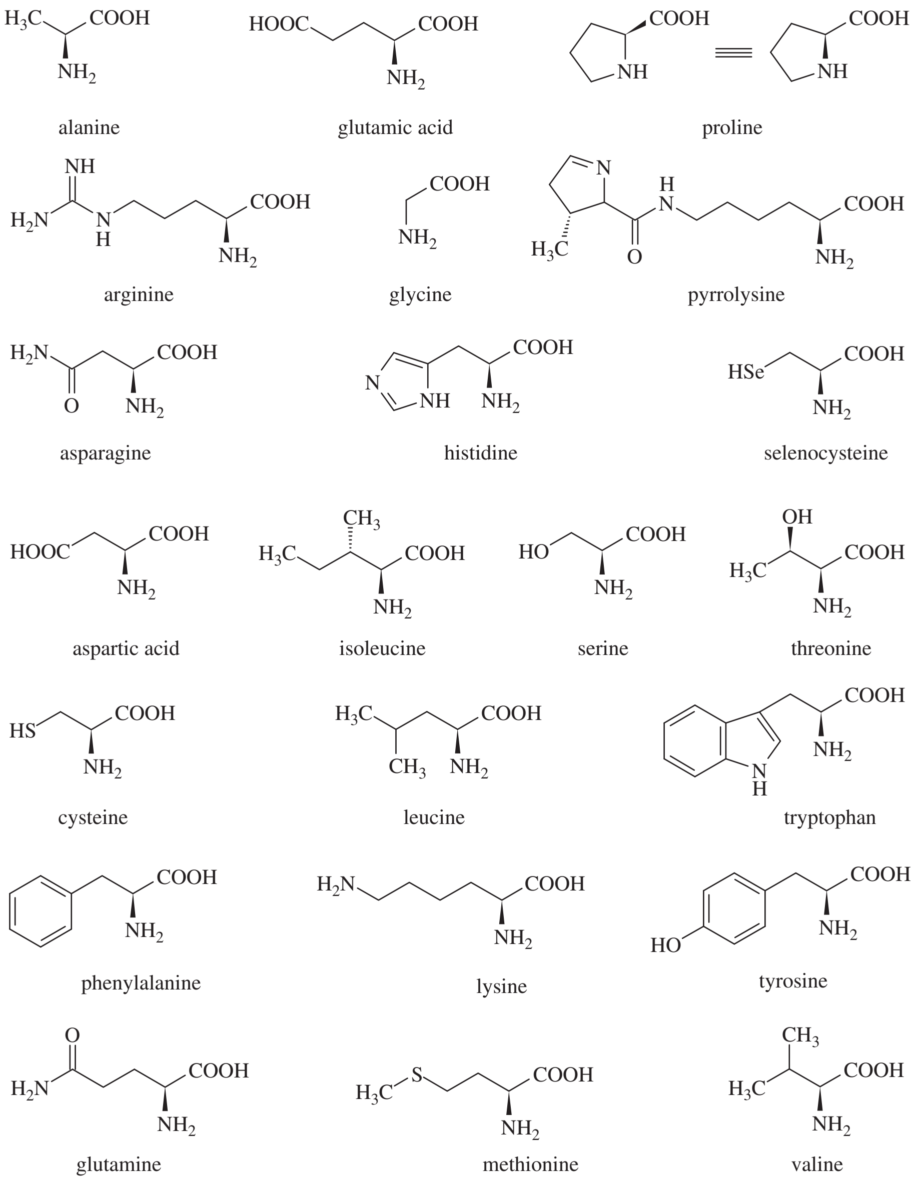
- **Histidine** Common proteins contain 2–3% histidine (average content is 2.1%), and blood plasma proteins contain up to 6% histidine. The flesh of some fish (especially mackerel and tuna) contains from 0.6 to 1.3% (and sometimes more than 2%) of free histidine. The free histidine content in the flesh of other fish is only 0.005–0.05%.

Non-essential amino acids

- **Glycine** Glycine is contained in a significant amount (25–30%) mainly in the structural protein collagen and in gelatine; in the majority of albumins it is not present at all (average content is 7.5%).

- **Alanine** Alanine occurs in almost all proteins in amounts of 2–12% (average content is 9.0%). Maize prolamine protein zein and animal protein gelatine contain about 9% of alanine.

- **Serine** Serine is found in many proteins; generally in an amount of 4–8% (average content is 7.1%).



2-4, basic amino acids

- **Cysteine** The highest amount of this amino acid and its oxidation product cystine (**2-5**) is present in keratin (up to 17%); it occurs in many other proteins in smaller amounts (1–2%). The average content is 2.8%. In the organism, cysteine can partially replace the essential amino acid methionine.

- **Aspartic acid and asparagine** The average content of aspartic acid in proteins is 5.5%; the average content of asparagine is 4.4%. Aspartic acid is the major amino acid of animal proteins known as globulins and albumins (6–10%). Vegetable proteins contain 3–13% aspartic acid, mainly in the form of asparagine (e.g. wheat proteins contain about 4% and maize proteins about 12%).

- **Glutamic acid and glutamine** The average content of glutamic acid and glutamine in proteins is 6.2 and 3.9%, respectively. Glutamic acid is the most abundant amino acid in the nervous tissue. In conventional proteins, both amino acids are usually found in larger quantities (especially in globulins) in cereal and legume proteins (18–40%). Wheat gluten (in its component gliadin) contains about 40%, soy protein contains about 18% and milk proteins contain about 22% of glutamic acid.

- **Selenocysteine** In most foods of both vegetable and animal origin, selenocysteine is the main form of selenium bound in proteins. The content of this amino acid, as well as the contents of other amino acids and peptides containing selenium (L-selenocystine, *Se*-methyl-L-selenocysteine, L,L-selenocystathionine, L-selenomethionine and γ-glutamyl-*Se*-methyl-L-selenocysteine), is unknown. Selenocysteine is typically located in a small number of active centres of proteins of Archaea, bacteria and eukaryotes (in glutathione

peroxidase, thioredoxin reductase, formiate dehydrogenase, glycine reductase and in some hydrogenases). Usually only one molecule of selenocysteine is bound in the peptide chain; however proteins containing multiple molecules of this amino acid are also known. For example, the isoenzyme of mammalian glutathione peroxidase, known under the abbreviation GPx6, is a selenoprotein in humans with cysteine-containing homologues in rodents.

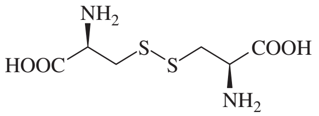
- **Tyrosine** Tyrosine accompanies phenylalanine in most proteins in an amount of 2–6% (average content is 3.5%). Gelatine contains only traces of tyrosine.

- **Proline** Proline is present in most proteins in amounts of 4–7%; the average content is 4.6%. Its content in the component of wheat gluten, gliadin, is about 10%, and about 12% of proline contains casein. Proline content in gelatine can be up to 13%. In bacteria, plants and animals it also plays a role as an osmoprotectant that helps stabilise proteins and cell membranes from the damaging effect of high osmotic pressure.

2.2.1.1.2 Modified proteinogenic amino acids

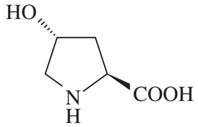
Post-translational modification, which extends the range of functions of the protein, is one of the later steps in the biosynthesis of many proteins. Post-translational modification of proteins occurs by methylation, hydroxylation, acetylation and phosphorylation of the protein functional groups, by attaching various lipids and carbohydrates to the protein molecule and by making structural changes such as the formation of disulfide bonds from cysteine residues by oxidation.

Post-translational oxidation of the thiol groups of cysteine residues in proteins yields L-**cystine** (Cys-Cys, **2-5**). The disulfide bond (or bridge) plays an important role in the structure of many proteins, as it combines two different polypeptide chains or two molecules of cysteine in the same peptide chain. For example, the major globular protein of milk β-lactoglobulin contains two disulfide bridges, and the glycoprotein of egg white ovomucoid has three domains connected by disulfide bridges. The protein keratin has a high cystine content: for example, human hairs contain about 5% cystine.



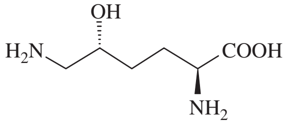
2-5, cystine

Another common post-translationally modified amino acid is the proline derivative L-4-**hydroxyproline**, (2*S*,4*R*)-4-hydroxyproline, also known as L-4-hydroxypyrrolidine-2-carboxylic acid (abbreviated Hyp, **2-6**), which is an important structural component of collagen, gelatine (about 12% of content) and the polypeptide (glycopeptide) of plant cell walls known by the trivial name extensin (see Section 4.5.1.3.1). Its content is low in most other proteins. The amount of 4-hydroxyproline in meat products therefore correlates with the lower quality of raw material used, for instance where the products contain skin, wherein collagen is the major protein. 4-Hydroxyproline also occurs in smaller amounts in plants. For example, concentrations ranging from 1.0 to 4.2 mg/kg have been found in the edible part of bergamot fruits (*Citrus bergamia*, Rutaceae).



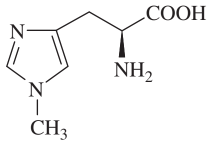
2-6, 4-hydroxyproline

4-Hydroxyproline in collagen is accompanied by a small amount of its isomer, L-3-hydroxyproline (L-3-hydroxypyrrolidin-2-carboxylic acid) and by a hydroxy derivative of lysine, also known as L-5-**hydroxylysine**, (2*S*,5*R*)-5-hydroxylysine, L-5-hydroxy-2,6-diaminohexanoic acid (abbreviated Hyl, **2-7**). In glycoproteins, hydroxylysine is bound as *O*-glycoside. Small amounts of hydroxylysine occur in plant materials (as a free amino acid in alfalfa forage, *Medicago sativa*, Fabaceae).



2-7, 5-hydroxylysine

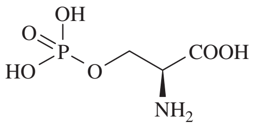
The minor amino acid typically present in the meat myofibrillar protein actin (also in some myosin isoforms and in dipeptide anserine, see Section 2.3.3.1.3) is L-3-**methylhistidine** (**2-8**), which is formed by methylation of histidine bound at the 73rd position of the protein chain. The functional significance of this modification of actin is not known; it is probably related to the metabolism of phosphates, with which the side chain of methylhistidine interacts. Methylhistidine does not occur in other protein-rich foods such as milk, eggs and soybeans. Its contents might therefore serve as a criterion for determining the quality ingredients in meat products.



2-8, 3-methylhistidine

The other amino acid that is usually modified is L-serine. It can be esterified with phosphoric acid. This ester, *O*-**phosphoserine** (**2-9**), occurs in many proteins, such as glycophosphoprotein phosvitin (also known as phosphovitin) from egg yolk (see Section 2.4.5.3.2). Phosvitins are one of the most phosphorylated (10%) proteins in nature, and are important for sequestering cations of calcium, iron and other metals for the developing embryo. Serine in phosvitin represents nearly 50% of the amino acids and there

are about 90% of the molecules of serine phosphorylated. Phosphoserine also occurs in the glycoprotein α_{S1}-casein (eight phosphoserine residues) and β-casein (five phosphoserine residues). Phosphoserine (as phospatidyl-L-serine) is also a component of phospholipids distributed widely among animals, plants and microorganisms. Phosphoserine usually constitutes less than 10% of the total phospholipids, the greatest concentration being in myelin from brain tissue and in wheat germ. Threonine residues in proteins are also esterified fairly frequently with phosphoric acid yielding *O*-**phosphothreonine**. Phosphatidyl-L-threonine was first detected in animal brains and tuna muscle, before it was characterised definitively as a minor component of neurons, macrophages, viruses and some bacterial species.



2-9, *O*-phosphoserine

2.2.1.2 Other amino acids

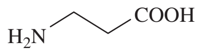
Plants and microorganisms are able to generate all 21 amino acids necessary for protein synthesis, and they can additionally synthesise many more. Foods contain numerous other less common amino acids in addition to those that are constituents of proteins (proteinogenic) and modified proteinogenic amino acids. It is estimated that there are around 700 amino acids known in nature, of which at least 300 are found in plants. They are often bound in peptides (see Section 2.3) or are present as free amino acids. In biochemistry, these **non-protein amino acids** are often classified as the so-called secondary metabolites, as they are the products of three major routes: modification of an existing (often proteinogenic) amino acid, modification of an existing pathway and by novel pathways. These amino acids become the biosynthetic precursors of many biologically active nitrogenous compounds, such as signalling molecules, vitamins, alkaloids (such as valine, leucine, isoleucine, threonine, arginine, lysine, phenylalanine and tryptophan), bile acids and pigments. Some of these amino acids also have specific functions in organisms; they act as neurotransmitters, stimulants and hormones. For example, phenylalanine (or 3,4-dihydroxyphenylalanine) is a precursor of adrenal hormones catecholamines (such as epinephrine, which is also known as adrenaline; see Section 10.3.2.10.1) and of the thyroid hormone thyroxine (see Section 2.2.1.2.5). Other amino acids increase plant tolerance to abiotic stress factors, act as toxic substances which protect plants against invading viruses, microorganisms, other plants and predators (e.g. the arginine analogue canavanine found in some legumes) and also serve as a storage and transport form of nitrogen and sulfur. Some of the unusual amino acids form secondarily during storage of raw materials and food processing via the activities of microorganisms and by chemical transformation of proteins (e.g. lysinoalanine, see **2-121**), peptides or free amino acids.

The following sections of this chapter present some of the most important non-protein amino acids in foods and feeds. For clarity, they are sorted according to the structure in which they are present in living organisms and not by function (these functions often are not well known).

2.2.1.2.1 Neutral aliphatic and alicyclic amino acids

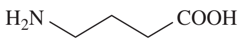
3-Amino acids and 4-amino acids

In addition to α-amino acids, β- and γ-amino acids can also be found in food. Naturally occurring β-alanine (3-aminopropionic acid, **2-10**) acts in the biosynthesis of pantothenic acid, acetyl coenzyme A, other acyl coenzymes A (see Section 5.9.1) and some histidine dipeptides. β-Alanine is synthesised in several different ways, by aspartic acid decarboxylation (in bacteria), but also by the transformation of propionic acid (in bacteria and some plants), by degradation of the polyamines spermine and spermidine (in yeasts and many plants, such as tomato, soybean and maize), or arising from the pyrimidine derivative uracil that occurs in all animals and in some plants, such as wheat.



2-10, β-alanine

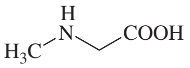
The higher homologue of β-alanine is γ-aminobutyric acid (4-aminobutanoic acid), known under the acronym of GABA (**2-11**), which is formed almost exclusively by enzymatic decarboxylation of glutamic acid. It is mainly present in the brain tissue of animals, where it acts as an inhibitor of nerve impulse transmissions. It is also found in plants and microbial cells, where it has a role as a signal molecule, and is also a constituent of some peptides such as nisine (see Section 11.2.1.2.1).



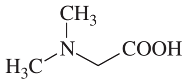
2-11, γ-aminobutyric acid

N-Substituted amino acids

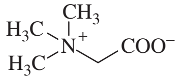
N-Alkylsubstituted amino acids are commonly found in foods. The simplest of these amino acids is *N*-methylglycine (sarcosine, **2-12**), which is a product of the catabolism of choline (see Section 3.5.1.1.1), creatine (see Section 2.2.1.2.4) or forms by *N*-methylation of glycine. Sarcosine probably has a regulatory function in methylation reactions. The subsequent *N*-methylation of sarcosine gives *N*,*N*-dimethylglycine (**2-13**), which is a component of pangamic acid ranked first among the vitamins (see Section 5.15). *N*,*N*,*N*-Trimethylglycine (*N*,*N*,*N*-trimethylammonioacetate, **2-14**) was named glycinebetaine or just betaine after its discovery in sugar beet (*Beta vulgaris*, Amaranthaceae), where it occurs in the amount of about 2.5 g/kg in the root. Glycinebetaine is a very effective osmolyte of microorganisms and higher plants, which results from choline or by *N*-methylation of glycine and sarcosine, respectively, and acts in various transmethylation reactions. It is present in higher amounts in sugar beets and molasses. Glycinebetaine was found to accumulate at high levels in some higher fungi. For example, its content in horse mushroom (*Agaricus arvensis*) is 7.8% dry matter.



2-12, *N*-methylglycine (sarcosine)

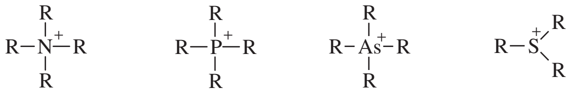


2-13, *N*,*N*-dimethylglycine



2-14, *N*,*N*,*N*-trimethylglycine

The terms **betaines** include glycinebetaine and similar zwitterionic ammonium compounds derived from other amino acids. By extension, betaines are also neutral molecules having charge-separated forms with an onium atom which bears no hydrogen atoms (such as phosphonium, sulfonium and arsonium ions) that is not adjacent to the anionic atom. Examples of the most common onium ions (ammonium, phosphonium, sulfonium and arsonium ions) are given by the general formulae **2-15**. The attached atoms are typically organic substituents, for example methyl groups. The most common betaines are quaternary ammonium compounds that occur in foods of animal and plant origin. In animals, betaines participate (along with *S*-adenosyl-L-methionine, pyridoxine, folic acid and vitamin B_{12}) in the protection of the cardiovascular system by lowering the potentially toxic concentrations of L-homocysteine. L-Carnitine plays an important role in the metabolism of fatty acids in biological systems plays (see Section 5.15). An arsenic analogue of betaine, arsenobetaine, accumulates in the body of some fish (see Section 6.2.3.1). Betaines are particularly ubiquitous in plants. They are generally referred to as osmolytes as they, like their amino acid precursors, tend to accumulate in the cytoplasm and intercellular fluids, where they exert protective functions for proteins, nucleic acids and cell membranes in response to abiotic stresses, such as cold, freezing, high temperature, the presence of toxic metals, reduced availability of water and high salinity. Some of the major betaines are listed in Table 2.2. Their structures are given by formulae **2-16**.



ammonium ion phosphonium ion arsonium ion sulfonium ion

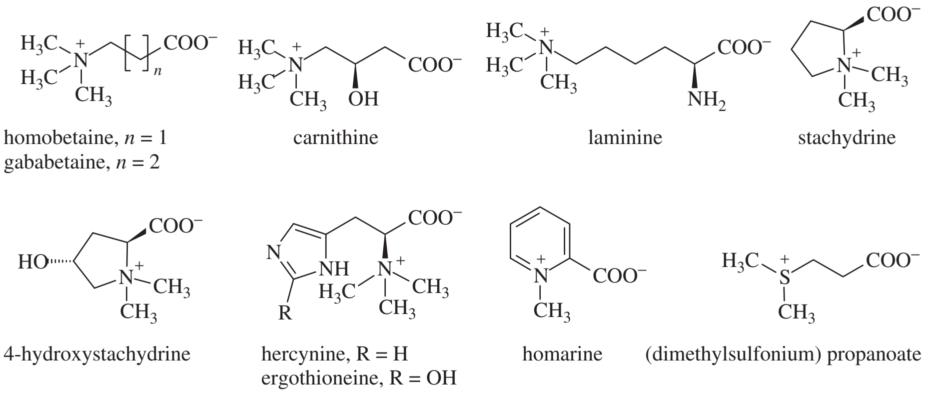
2-15, general structures of some onium ions

An important group of *N*-substituted amino acids are *N*-acylamino acids (**2-17**). An important metabolite is hippuric acid, also known as *N*-benzoylglycine (**2-17**). Hippuric acid arises from glycine as a result of detoxification of benzoic acid and other aromatic acids in humans and animals. In higher concentrations it occurs in the urine of herbivores and reportedly inhibits some pathogenic bacteria in the urinary tract. Benzoic acid is found (usually in small amounts) in many plant materials, including fruit, vegetables and forage crops (see Section 8.2.6.1.6), and is also used as a food preservative (see Section 11.2.1.1.1). Cows partially excrete hippuric acid into their milk, up to a concentration of 60 mg/kg. Microorganisms used in the production of fermented dairy products hydrolyse hippuric acid to glycine and benzoic acid. For example, the content of benzoic acid in yoghurts can reach, on average, 15 mg/kg.

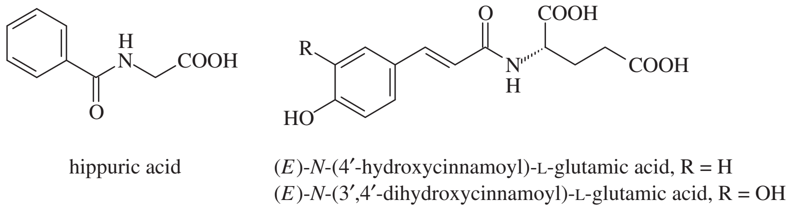
N-phenylpropenoyl amino acids (**2-17**) have been identified as the key contributors to the astringent taste of non-fermented cocoa beans and cocoa products. Besides the already known (*E*)-*N*-[3′,4′-dihydroxycinnamoyl-3-hydroxy-L-tyrosine (known as clovamide), (*E*)-*N*-(4′-hydroxycinnamoyl)-L-tyrosine (deoxyclovamide) and (*E*)-*N*-(3′,4′-dihydroxycinnamoyl)-L-tyrosine, seven additional amides derived from cinnamic, 4-coumaric, caffeic and ferulic acids, namely, (+)-(*E*)-*N*-(cinnamoyl)-L-aspartic acid, (+)-(*E*)-*N*-(4′-hydroxycinnamoyl)-L-aspartic acid, (+)-(*E*)-*N*-(3′,4′-dihydroxycinnamoyl)-L-aspartic acid, (+)-(*E*)-*N*-(4′-hydroxy-3′methoxycinnamoyl)-L-aspartic acid, (−)-(*E*)-*N*-(4′-hydroxycinnamoyl)-L-glutamic acid, (−)-(*E*)-*N*-(3′,4′-dihydroxycinnamoyl)-L-glutamic acid and (−)-(*E*)-N-(4′-hydroxycinnamoyl)-3-hydroxy-L-tyrosine, were recently identified.

Table 2.2 Some important betaines.

Betaine	Initial amino acid	Occurrence
β-Alaninebetaine (homobetaine)	β-Alanine	Plants of the Plumbaginaceae family, citrus species, meat
Gababetaine	γ-Aminobutyric acid	Molluscs, citrus species
L-Carnitine	Lysine and methionine	Meat, dairy products, legumes, vegetables, higher fungi
Laminine (lysinebetaine)	Lysine	Some higher plants
Stachydrine (cadabine, prolinebetaine)	Proline	Higher plants (e.g. citrus species), fungi
Betonicine (4-hydroxystachydrine, 4-hydroxyprolinebetaine)	4-Hydroxyproline	Plants of the Lamiaceae family
Hercynine (histidinebetaine)	Histidine	Higher fungi, citrus species
L-Ergothioneine (2-thioimidazolebetaine)	2-Mercaptohistidine	Higher fungi, plants
Homarine	Pyridine-2-carboxylic acid	Some higher plants
(Dimethylsulfonium)propanoate	Methionine	Marine phytoplankton, seaweeds



2-16, structures of important betaines



2-17, structures of important *N*-acylamino acids

Exposure to light rapidly converted (*E*)-isomers of *N*-phenylpropenoyl amino acids into the corresponding (*Z*)-isomers.

Alicyclic amino acids

A common neutral amino acid derived from cyclopropane is 1-aminocyclopropane-1-carboxylic acid. The precursor of this carboxylic acid is methionine, or strictly *S*-adenosyl-L-methionine which is derived from methionine (Figure 2.1). 1-Aminocyclopropane-1-carboxylic acid is also present in apples, pears and other fruits. 1-Aminocyclopropane-1-carboxylic acid serves as a precursor of ethylene (ethene) in essentially all tissues of vascular plants and also in fungi and algae. Ethylene is a signalling molecule in plants (a hormone) that regulates seed germination, plant growth, flowering, ripening of fruits, leaf abscission and aging. Plants also synthesise ethylene in various stressful situations (mechanical damage, frost damage, flooding, pathogen attack and in the presence of heavy metals). Production of ethylene is connected with the formation of hydrogen cyanide, a process known as cyanogenesis (see Section 10.3.2.3).

The seeds of lychee fruit (*Litchi chinensis*) from the soapberry family (Sapindaceae), native to southern China and South-east Asia, contains an unusual amino acid L-α-(methylenecyclopropyl)glycine, that is, (2*S*,3*S*)-2-(methylenecyclopropyl)

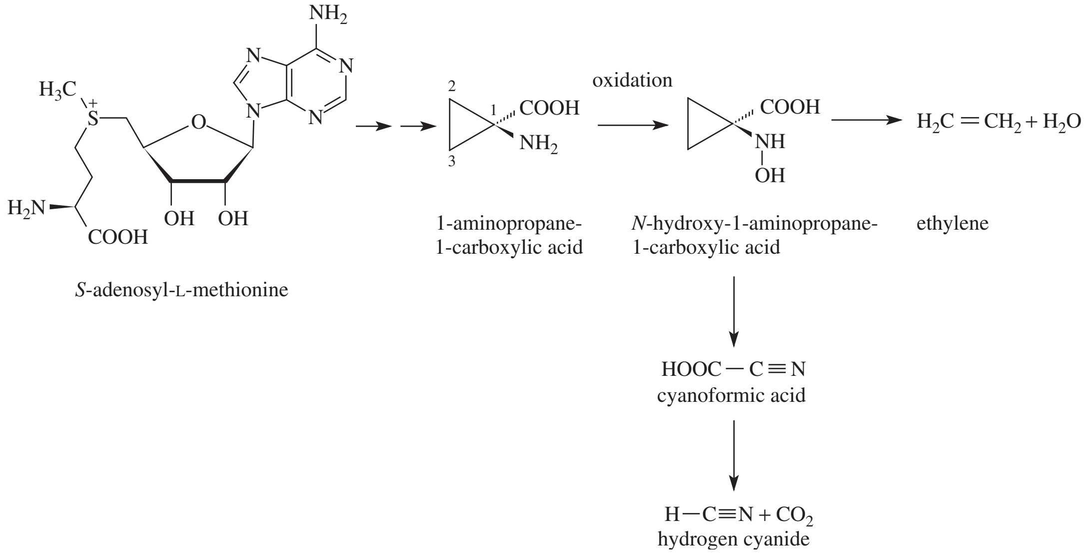
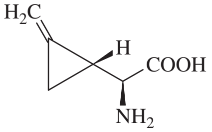


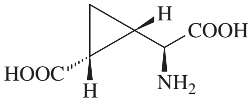
Figure 2.1 Formation of ethylene and hydrogen cyanide in plants.

glycine (**2-18**), which lowers blood glucose levels. Symptoms of poisoning, known as toxic hypoglycaemic syndrome, occur 6–48 hours after ingestion of seminal immature follicles. The poisoning is manifested by vomiting, drowsiness, fatigue and hypoglycaemia. The toxin (the active metabolite is methylencyclopropylacetyl-CoA) interferes with the metabolism of branched-chain amino acids, irreversibly binds to FAD and inhibits acyldehydrogenases acting in β-oxidation of fatty acids.

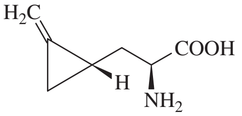


2-18, 2-(methylenecyclopropyl) glycine

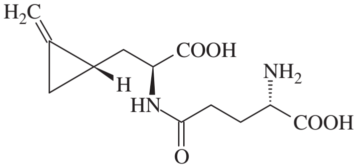
The same amino acid, together with derived γ-glutamyl dipeptide and (2*S*,1′*S*,2′*S*)-2-(2′-carboxycyclopropyl)glycine (**2-19**) occurs in the seeds of ackee fruit (*Blighia sapida*, Sapindaceae), native to tropical West Africa. A higher homologue of 2-(methylenecyclopropyl)glycine, that is, L-3-(methylenecyclopropyl)alanine, also known as hypoglycin A (**2-19**), is present in this fruit in much higher concentrations. Hypoglycin A (now known as hypoglycin) occurs as a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-diastereoisomers in fruits (in the edible arilli portion) and seeds. The former is the dominant isomer in the arilli. Formula (**2-20**) shows the (2*S*,4*S*)-isomer, which also has a hypoglycaemic effect, but the other primary toxic effect is known as Jamaican Vomiting Sickness (see Section 10.3.2.7). Ackee fruit also contains the γ-glutamyl dipeptide of hypoglycin A, known as hypoglycin B (**2-21**). The amount of hypoglycin A in totally green and yellow–green or orange–green arilli and seeds is 6.7–7.9 and 8.2–8.4 g/kg, respectively. Dull and shrivelled arilli (mainly from red fruits) and seeds contain 0.27–0.55 and 1.5–2.6 g/kg of hypoglycin A, respectively. The content of hypoglycin B in the seeds of unripe fruit is 1.6–3.1 g/kg, while in seeds of ripe fruit the content ranges from 11.7 to 12.6 mg/kg. Higher concentrations of hypoglycins A and B also occur in seeds of the common sycamore (*Acer pseudoplatanus*, Aceraceae). For example, The Food and Drug Administration and Health Canada sets the maximum permissible level of hypoglycin A to 100 mg/kg.



2-19, 2-(2′-carboxycyclopropyl)glycine

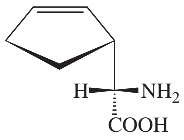


2-20, hypoglycin A

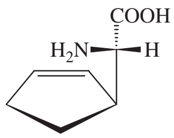


2-21, hypoglycin B

An important alicyclic amino acid in some genera of tropical plants of the Flacourtiaceae, Passifloraceae and Turneraceae families is cyclopentenylglycine occurring as a mixture of two stereoisomers, (2*S*,1′*R*)-2-(cyclopent-2′-en-1′-yl)glycine (**2-22**) and (2*S*,1′*S*)-2-(cyclopent-2′-en-1′-yl)glycine (**2-23**), in which the (2*S*,1′*R*)-isomer predominates. Cyclopentenylglycine is a precursor of unusual fatty acids (see Section 3.3.1.4.3) and also cyanogenic glycosides (see Section 10.3.2.3.1).



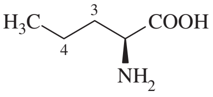
2-22, (2*S*,1′*R*)-2-(cyclopent-2′-en-1′-yl)glycine



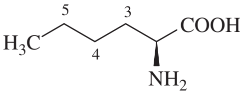
2-23, (2*S*,1′*S*)-2-(cyclopent-2′-en-1′-yl)glycine

Hydroxyamino acids

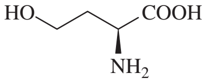
Various legumes (seeds of plants of the Fabaceae family) are rich sources of free aliphatic hydroxyamino acids. Common amino acids such as 4-hydroxyleucine, 4-hydroxynorvaline and 5-hydroxynorleucine are hydroxyderivatives of branched chain amino acids, L-leucine (2-4), L-isoleucine (**2-4**), L-norvaline (**2-24**) and L-norleucine (**2-25**). Another common amino acid in legumes is L-homoserine (**2-26**), which is derived from 2-aminobutyric acid, the intermediate in the biosynthesis of threonine from aspartic acid. Frequently *O*-acyl- and *O*-amino derivatives of homoserine are seen. An example of an *O*-aminoderivative of homoserine is a toxic amino acid known as canaline (see Section 2.2.1.2.4). The most common source of this amino acid is the jack bean (*Canavalia ensiformis*).



2-24, norvaline



2-25, norleucine



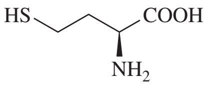
2-26, homoserine

These hydroxyamino acids are frequent components of peptides occurring in toxic mushrooms. For example, peptides of some *Amanita* species (see Section 10.3.2.9.2) contain (2*S*,4*R*)- and (2*S*,4*S*)-isomers of 4-hydroxyleucine and some other hydroxysubstituted amino acids, including amino acids with unsaturated side chains.

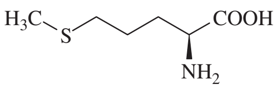
2.2.1.2.2 Sulfur and selenium amino acids

Foods of plant origin contain numerous sulfur amino acids, mostly derived from cysteine, methionine and their higher homologues,

such as L-homocysteine (**2-27**) and L-homomethionine, also known as L-5-methylthionorvaline (**2-28**).



2-27, homocysteine

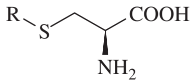


2-28, homomethionine

Cysteine derivatives

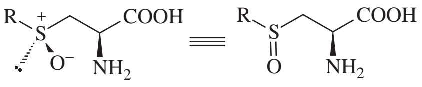
Plants synthesise a number of *S*-cysteine (and *S*-glutathione) conjugates that are released by the action of L-cysteine-*S*-conjugate thiol-lyases (deaminating) to produce various volatile flavour-active thiols in fruits and vegetables (see Section 8.2.9.1.2).

Other important derivatives of cysteine are *S*-alk(en)yl-L-cysteines (**2-29**), which accompany, in small amounts, the predominant *S*-alk(en)yl-L-cysteine *S*-oxides, also called *S*-alk(en)yl-L-cysteine sulfoxides (**2-30**). *S*-Alk(en)yl-L-cysteine sulfoxides may occur in two optical isomers (thanks to the free electron pair on the sulfur atom). Only (+)-*S*-isomers, also known as (S_S)-isomers, occur in vegetables. However, isoalliin is an exception. It occurs as an (R_S)-isomer because of the priority of the other substituent. The bond between the sulfur and oxygen atoms in *S*-alk(en)yl-L-cysteine sulfoxides differs from a conventional double bond between carbon and oxygen. As a result, the molecule has a dipolar character, with the negative charge centred on the oxygen and the sulfur atom being a chiral centre. These amino acids originate from γ-glutamyl *S*-alk(en)yl-L-cysteine storage compounds via *S*-alk(en)yl-L-cysteines (see Section 2.3.2.1.2) and are the precursors of many biologically and flavour active compounds (see Section 8.2.9.1.4). Particularly rich sources of these amino acids are some genera of the plant families Brassicaceae (the genus *Brassica*), Amaryllidaceae (subfamily Allioideae, genera *Allium* and *Tulbaghia*) and Fabaceae (*Phaseolus*, *Vigna* and *Acacia*). *S*-Alk(en)ylcysteine derivatives also occur in plants belonging to the families Olacaceae (*Scorodocarpus*) and Phytolaccaceae (*Petiveria*), in some higher fungi, such as mushrooms in the genera *Marasmius*, *Collybia* and *Lentinula* (known as shiitake), and in brown (*Undaria*) and red (*Chondria*) seaweeds.

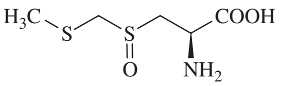


2-29, *S*-alk(en)ylcysteines

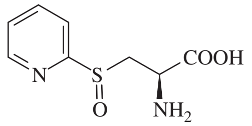
The basic member of the homologous series, *S*-methylcysteine (**2-29**, R = CH_3), and its sulfoxide, (R_C,S_S)-*S*-methylcysteine sulfoxide, also known as (+)-*S*-methyl-L-cysteine sulfoxide or methiin (**2-30**, R = CH_3), occurs in cabbage, garlic, onion and other *Brassica* and *Allium* vegetables and also in beans. There is roughly 0.2–0.7 g/kg in fresh Brassica vegetables. The methiin levels in *Allium* vegetables are given in Table 2.3. Note that methiin is a toxic amino acid to ruminants (see Section 10.3.2.7.1). (R_C,S_S)-*S*-Carboxymethylcysteine sulfoxide (**2-30**, R = CH_2–COOH), occurs, for example, in radish (genus *Raphanus*, Brassicaceae). Some species of *Brassica* plants and some members of the genus *Allium* (e.g. elephant garlic, onion, shallot, leek and chive) contain traces of ethiin, that is, (R_C,S_S)-*S*-ethylcysteine sulfoxide (**2-30**, R = CH_2–CH_3) (Table 2.3).



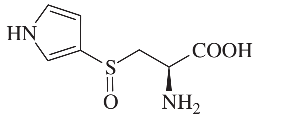
alk(en)ylcysteine sulfoxides



marasmin



S-(pyridin-2-yl)cysteine sulfoxide



S-(pyrrol-3-yl)cysteine sulfoxide

2-30, *S*-substituted L-cysteine sulfoxides

Table 2.3 Concentrations of alke(en)yl-L-cysteine sulfoxides in *Allium* vegetables.

	Concentration in g/kg fresh weight			
Substituent	**Garlic (*Allium sativum*)**	**Onion (*Allium cepa*)**	**Leek (*Allium porum*)**	**Chive (*Allium schoenoprasum*)**
Methyl	0.05–4.24	0.22	0.04	0.32
Ethyl	0–traces	0.05	traces	0.01
Propyl	0–traces	0.06	traces	0.07
Prop-2-en-1-yl (allyl)	1.10-10.5	traces–0.05	traces	0.02
Prop-1-en-1-yl	traces–1.11	0.50–1.31	0.18	0.31

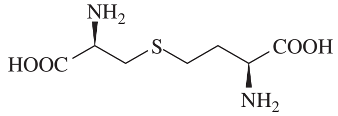
Another related amino acids are (R_C,S_S)-*S*-allylcysteine (deoxyalliin), and (R_C,S_S)-*S*-(prop-2-en-1-yl)cysteine sulfoxide, which is known as alliin (**2-30**, R=CH_2–CH=CH_2). Alliin is the major sulfur containing amino acid of garlic (Table 2.3). Its isomer (R_C,R_S,1*E*)-*S*-(prop-1-en-1-yl)cysteine sulfoxide, or isoalliin (**2-30**, R=CH=CH–CH_3), is the major free amino acid of onions. It is also found in smaller amounts in garlic (Table 2.3). (R_C,S_S)-*S*-Propylcysteine sulfoxide, or propiin, R = $[CH_2]_2$–CH_3 (**2-30**) occurs in onions, shallots and chives and in trace amounts in some garlic species, for example in bulbs of *A. vineale* (0.01 mg/kg fresh weight), *A. ursinum* (0.02 mg/kg fresh weight) and *A. triquetrum* (0.09 mg/kg fresh weight). Its higher homologue butiin, that is, (R_C,S_S)-*S*-butylcysteine sulfoxide, R=$[CH_2]_3$–CH_3 (**2-30**), occurs in some species of the genus *Allium*, in bulbs of *A. siculum* (0.3 g/kg fresh weight) and *A. tripedale* (5.7 g/kg fresh weight) together with (R_C,S_S,1*E*)-*S*-(but-1-en-1-yl)cysteine sulfoxide, R=CH=CH–CH_2–CH_3 (**2-30**), known as homoisoalliin. The concentrations found in fresh bulbs of the ornamental plants *A. siculum* and *A. tripedale* were 3.5 and 1.2 g/kg, respectively. The important cysteine sulfoxide (R_S,R_C)-*S*-(methylthiomethyl)cysteine-4-oxide, known as marasmin (**2-30**), carrying a methylthiomethyl moiety as the aliphatic residue, was found in South African society garlic (*Tulbaghia violacea*) and in wild (woodland) garlic (*T. alliacea*), in *A. stipitatum* and *A. suworowii* (*Allium* subgenus *Melanocrommyum*) and as a γ-glutamyl derivative, γ-glutamyl-(S_S,R_C)-marasmin, in various mushroom species belonging to the genus *Marasmius* (such as *M. alliaceus*). Marasmin is the precursor of the thiosulfinate marasmicin, which shows antifungal and tuberculostatic activities. (R_C,S_S)-*S*-(Pyridin-2-yl)cysteine sulfoxide (**2-30**) and several pyridyl compounds, which were formed from this amino acid by the action of alliinase, were identified in bulbs of the drumstick onion *Allium stipitatum* (*Allium* subgenus *Melanocrommyum*), which is used as a crop plant and in folk medicine in Central Asia. (R_C,S_S)-*S*-(Pyrrol-3-yl)cysteine sulfoxide is found in another member of the subgenus *Melanocrommyum*, *A. giganteum*.

To date, six *S*-alk(en)ylcysteine sulfoxides, namely *S*-allyl-, (*E*)-*S*-(prop-1-en-1-yl)-, *S*-methyl-, *S*-propyl, *S*-ethyl and *S*-butylcysteine sulfoxides have been found in common vegetables of the genus *Allium*; the last two compounds are only present in trace amounts. It has been well documented that the total content and relative proportions of the individual *S*-alk(en)ylcysteine sulfoxides in alliaceous plants are significantly affected by a number of genetic and environmental factors (e.g. plant species and variety, climatic conditions, sulfur content in the soil, irrigation, fertilisation, harvest date, amongst other things) and is usually around 0.1–0.8%, which represents more than 10% of the crude protein content. The contents also vary considerably over the growth period. For example, in garlic, the highest amounts are found in spring, in the green parts of the plant, which are the most important sites of cysteine sulfoxide biosynthesis, although some biogenesis may also occur in the bulbs. The cysteine sulfoxide levels in the bulbs start to increase dramatically approximately 5 weeks before harvest. This accumulation in bulbs may be associated with the possible role of cysteine sulfoxides as storage compounds for sulfur and nitrogen during dormancy.

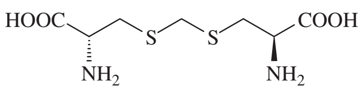
Some vegetables also contain alk(en)ylthio substituted L-cysteine sulfoxides in small amounts. For example, (R_C,S_S)-*S*-methylthiocysteine sulfoxide (R=S–CH_3, **2-30**), (R_C,S_S)-*S*-propylthiocysteine sulfoxide (**2-30**, R=S–CH_2–CH_2–CH_3) and (R_C,S_S,1*E*)-*S*-(prop-1-en-1-ylthiocysteine sulfoxide (**2-30**, R=S–CH=CH–CH_3) occur in onions at concentrations of 0.19, 0.01 and 0.56 g/kg fresh weight, respectively. These amino acids and the corresponding peptides (see Section 2.3.3.1.2) are formed by reaction of thiosulfinates (see Section 8.2.9.1.4) with cysteine residues.

An interesting sulfur containing amino acid derived from cysteine is 2-amino-4,6,8,10,10-pentaoxo-4,6,8,10-tetrathiaundecanoic acid (**2-30**, R=CH_2–CH_2-SO–SO–CH_2–SO_2–CH_3), or deglutamyllentinic acid), which forms from the corresponding γ-glutamyl peptide known as lentinic acid (see Section 2.3.2.1.2) through the action of γ-glutamyl transpeptidase and becomes the precursor of the characteristic sulfur compound (1,2,4,5,6-pentathiepane, commonly known as lenthionine; see Section 8.2.12.9.7) in dried edible shiitake mushrooms (*Lentinula edodes*), one of the most popular edible mushrooms in Japan and other parts of the Far East.

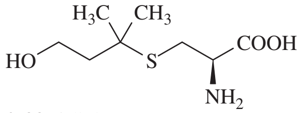
The unusual cysteine derivative L,L-cystathionine (**2-31**) is an intermediate of the biosynthesis of methionine and of other amino acids. The toxic amino acid L,L-djenkolic acid, also known as 3,3′-(methylenedithio)di-L-alanine, consists of two cysteine residues joined by a methylene group between the sulfur atoms (**2-32**). It was first identified in the urine of Indonesians consuming tropical legumes known as jengkol or jering (*Archidendron pauciflorum*, syn. *Pithecellobium lobatum*, Fabaceae). The amino acid L-felinine, 3-hydroxy-1,1-dimethylpropyl-L-cysteine (**2-33**), occurs in the urine of cats and other felines, and is a precursor of the putative cat pheromone 3-mercapto-3-methylbutan-1-ol, which has a practical use in marking territory. Cysteine (or cystine) is also the precursor of dehydroalanine (2-aminoacrylic acid), from which the amino acid lanthionine is formed (see Section 2.5.1.3.4), which is also a component of the microbial peptide nisin (see Sections 2.3 and 11.2.1.2.1). Dehydroalanine is a precursor of other unusual amino acids (such as lysinoalanine). Lysinoalanine and its analogues can be generated as cross-links on heating of proteins and, especially, in proteins after treatment in alkaline solutions (as occurs, for example, in soybean processing). Digestive enzymes do not hydrolyse these cross-links. Other sulfur-containing amino acids include *N*-acetyl-*S*-substituted cysteines (or mercapturates, **2-34**), which form in the body as products of detoxification in reactions of xenobiotics with glutathione and by hydrolysis of the resulting products.



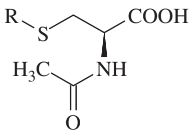
2-31, cystathionine



2-32, djenkolic acid



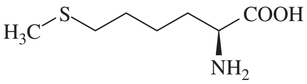
2-33, felinine



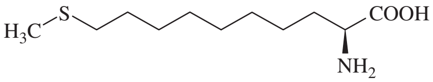
2-34, mercapturic acids

Methionine derivatives

A common amino acid in *Brassica* vegetables, *S*-methyl-L-methionine, was formerly classified as vitamin U (see Section 5.15). Higher homologues of methionine – L-homomethionine, L-dihomomethionine (**2-35**), L-trihomomethionine up to L-hexahomomethionine (**2-36**) – are starting compounds for the biosynthesis of many important glucosinolates (see Section 10.3.2.4.1). For example, homomethionine is the precursor of sinigrin and glucoibervirin, while dihomomethionine is the precursor of progoitrin and gluconapin.



2-35, dihomomethionine



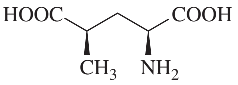
2-36, hexahomomethionine

Selenium amino acids

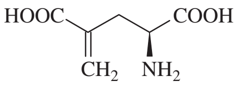
Allium vegetables and various other plants contain small amounts of amino acids derived from cysteine and methionine, in which sulfur is replaced by selenium. These amino acids are the main organic form of selenium and, together with *Se*-containing proteins, represent the main selenium source in foods (see Section 6.2.3.1). Examples of these amino acids are *Se*-alk(en)ylcysteines and their γ-glutamyl derivatives.

2.2.1.2.3 Acidic amino acids and their amides

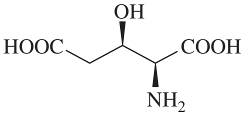
Most non-protein acidic amino acids and their amides are structurally related to L-glutamic acid and L-glutamine. For example, sea pea seeds (*Lathyrus maritimus*, Fabaceae) contain L-4-methylglutamic acid, that is, (2*S*,4*R*)-2-amino-4-methylpentanedioic acid (**2-37**), and the seeds of peanuts (*Arachis hypogaea*, Fabaceae) contain L-4-methyleneglutamic acid (**2-38**) together with L-4-methyleneglutamine. (2*S*,3*R*)-3-Hydroxyglutamic (*threo*-3-hydroxy-L-glutamic) acid (**2-39**) is a precursor of ibotenic acid and other toxic compounds in fly agaric (*Amanita muscaria*) and panther cap (*A. pantherina*). It is also a precursor of the tricholomic acid found in some agarics (*Tricholoma muscarium*) (see Section 10.3.2.9.3). The so-called false rhubarb (*Rheum rhaponticum*), red currant (*Ribes silvestre*, Grossulariaceae) and garden cress (*Lepidium sativum*, Brassicaceae) contain L-3,4-dihydroxyglutamic acid, that is, (2*S*,3*S*,4*R*)-2-amino-3,4-dihydroxypentanedioic acid. Plants also contain other stereoisomers of these amino acids and many other related compounds that exhibit a range of biological effects.



2-37, 4-methylglutamic acid

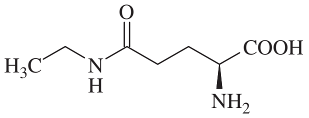


2-38, 4-methyleneglutamic acid



2-39, 3-hydroxyglutamic acid

N-Ethyl-L-glutamine, also known as L-theanine or L-ethanine (**2-40**), is a unique major free amino acid in tea leaves. Depending on the variety of Chinese tea (*Camellia sinensis*, Theaceae), its content in the leaves is normally around 1.3% of dry matter (0.7–2.0%) and is higher in green tea and tea of higher quality (0.8–2.7% in green tea, 0.6–2.1% in black tea, 2.0–9.2% in oolong tea, 0.7–1.8% in decaffeinated black tea). The tea extracts contain about 3% of theanine. The theanine content is used to detect tea extracts in products based on tea (instant and iced tea). Theanine has neuromuscular sedative effects with no side effects and is used in some special food supplements.

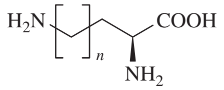


2-40, theanine

The fruiting bodies of common mushrooms *Agaricus bisporus* (also known as button mushrooms, white mushrooms, table mushrooms and champignon mushrooms) contain a mutagenic glutamic acid derived hydrazine β-*N*-(γ-L-glutamyl)-4-(hydroxymethyl)phenylhydrazine known as L-agaritine (10-226) at concentrations of 100–1 700 mg/kg. The metabolic fate of agaritine has been linked with the carcinogenity of this mushroom (see Section 10.3.2.11.4).

2.2.1.2.4 Basic amino acids and related compounds

Plants contain various basic diaminomonocarboxylic acids that are homologues of lysine or derivatives of these homologues. Other arginine derivatives and their homologues are also relatively common. The lowest member of the homologous series of compounds is L-2,3-diaminopropionic acid (**2-41**), occurring in plants of the genus *Mimosa* of the legume family Fabaceae. The toxic N^3-methyl derivative occurs in the cycads (plants of the cycas family Cycadaceae) and the N^2-oxalyl derivative is found in vetchlings (*Lathyrus* spp.) and vetches (*Vicia* spp.) that belong to the legume family Fabaceae. Together with L-2,4-diaminobutyric acid (**2-41**), this compound is the originator of neurodegenerative disease neurolathyrism (see Section 10.3.2.7.1).

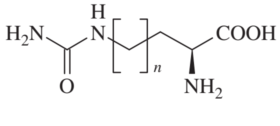


2-41, 2,3-diaminopropionic acid (*n* = 0)
2,4-diaminobutyric acid (*n* = 1)
2,5-diaminovaleric acid (ornithine, *n* = 2)

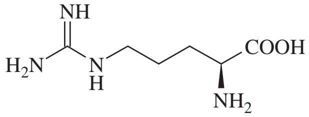
A common basic amino acid is the lower homologue of lysine, L-ornithine (L-2,5-diaminovaleric acid, **2-41**), which is an intermediate in the biosynthesis of arginine and an important amino acid of the ornithine (urea) cycle, which has the function of converting toxic ammonia into less toxic urea in mammals. Ornithine formed in dough through the action of yeast (*Saccharomyces cerevisiae*) is the main precursor of the typical aroma of bread crust, for which 2-acetyl-1-pyrroline together with 6-acetyl-1,2,3,4-tetrahydropyridine and its isomer 6-acetyl-2,3,4,5-tetrahydropyridine are responsible (see Section 8.2.12.4.1).

Reactions of L-lysine (**2-4**) with reducing sugars (known as the Maillard reaction) during the processing and storage of food produce the so-called bound lysine, which is present in the form of unavailable derivatives. Examples of these compounds are the amino acids furosine and pyridosine (see Section 4.7.5.12.3). Plants often contain derivatives of lysine, such as 4-hydroxylysine, which is found in some *Salvia* species of the mint family (Lamiaceae), commonly referred to as sage. N^6-Acetyllysine occurs in beet (*Beta vulgaris* group Altissima, Amaranthaceae, formerly Chenopodiaceae). The metabolic precursor of lysine, L-2-aminoadipic (α-aminoadipic) acid occurs in many legume seeds. For example, the concentration in lentils is 0.17 g/kg and in garden peas 0.18 mg/kg. Aminoadipic acid occurs in lower amounts in commercially available seedlings of leguminous plants sold for human consumption.

A non-protein amino acid related to arginine is the carbamoyl derivative of ornithine, L-citrulline (**2-42**), which is an intermediate in the urea cycle. It was first identified in watermelon (*Citrullus lanatus*, Cucurbitacaeae), where it is present at concentrations of 640–890 mg/kg fresh weight. In plants citrulline forms part of the nitrogen reserves and is a means of transporting nitrogen, as well as numerous amino acids and other nitrogenous compounds. It can also be formed by the decomposition of arginine, for example by yeast *Saccharomyces cerevisiae*. The seeds of some legumes, such as vetchlings (*Lathyrus* spp.) and vetches (*Vicia* spp.), contain its higher homologue, L-homocitrulline (**2-42**), and a number of amino acids containing the guanidino groups. Examples of these amino acids are L-4-hydroxyarginine, L-homoarginine (2-amino-6-guanidinohexanoic acid, **2-43**) and L-4-hydroxyhomoarginine. For example, homoarginine occurs in the seeds of red peas (*Lathyrus cicera*), grass peas (*L. sativus*) and in lentils (*Lens culinaris*).

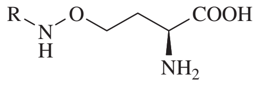


2-42, citrulline (*n* = 2)
homocitrulline (*n* = 3)

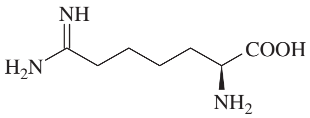


2-43, homoarginine

The subtropical legume jack bean (*Canavalia ensiformis*, Fabaceae), which is grown commercially as a source of the enzyme urease, contains two unusual amino acids derived from homoserine known as L-canaline, or L-2-amino-4-(aminooxy)butanoic acid, and L-canavanine, or L-2-amino-4-(guanidinooxy)butanoic acid (**2-44**). The canavanine concentration in seeds can be as high as 10% of dry matter. Toxic canaline, with a unique aminooxy group, is a structural analogue of ornithine and a lysine antagonist. Canavanine is a structural analogue of arginine and its highly potent antagonist (competitor) that blocks the binding of arginine (agonist) at a receptor molecule. By acting as an antagonist of arginine, canavanine inhibits the growth and development of other organisms and thus has an allelochemical effect. Toxicity is based upon its degradation to canaline (by arginase) and reaction of canaline with aldehydes, for instance with pyridoxal 5′-phosphate in molecules of decarboxylases and aminotransferases yielding stable oximes. Both amino acids show toxic effects to bacteria, insects, fungi, higher plants and predators. Canavanine has also been tested experimentally for cytotoxicity against cancer cells. A specific amino acid L-indospicine (**2-45**) is a hepatotoxin, which is present along with canavanine in seeds of the *Indigofera spicata* tree, which belongs to the same plant family (see Section 10.3.2.7.1). Other species of the genus *Indigofera* have found use as sources of a natural blue dye known as true indigo (*I. tinctoria* and *I. suffruticosa*), many *Indigofera* species have uses in traditional medicine and are also grown as ornamental plants.

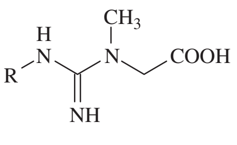


2-44, canaline, R = H
canavanine, R = C(=NH)NH_2



2-45, indospicine

The muscle tissues of warm-blooded animals and of fish contain an unusual amino acid, creatine phosphate (or phosphocreatine, **2-46**) in relatively high concentrations (3–6 g/kg). This acts as a phosphate donor (an energy reserve that can be rapidly mobilised) for anaerobic formation of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) in skeletal muscles and the brain following an intense muscular or neuronal effort. It is biosynthesised from glycine and arginine. Natural hydrolysis of phosphocreatine *post mortem* yields creatine (**2-46**), which occurs in raw meat. During thermal processing of meat and meat products, creatine dehydrates spontaneously to form creatinine (see **2-117**), which may become the precursor of toxic imidazopyridines, imidazoquinolines and imidazoquinoxalines in some processed meats and meat products (see Section 12.2.1). Crustaceans employ arginine instead of creatine. Creatine is often used as a food supplement for athletes and bodybuilders. It is claimed that it helps stimulate and maintain instantaneous muscle power.



2-46, creatine phosphate, R = PO_3H
creatine, R = H

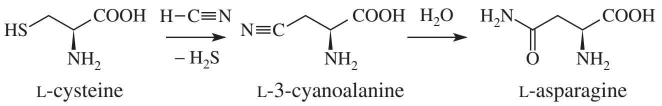


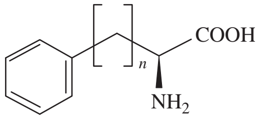
Figure 2.2 Formation of 3-cyanoalanine and asparagine.

Cyanoamino acids

The production of toxic hydrogen cyanide in plants, called cyanogenesis (see Section 10.3.2.3), requires effective mechanisms, which eliminate this toxic substance. One of these is the biosynthesis of neurotoxic β-substituted alanine derivative β-cyano-L-alanine (L-3-cyanoalanine) from cysteine, which takes place in all vascular plants under the catalysis of β-cyanoalanine synthase (Figure 2.2). β-Cyanoalanine is then converted into asparagine, which is involved in the normal metabolism. This reaction is catalysed by β-cyanoalanine hydratase.[1] Seeds of some vetches (*Vicia* spp.) and vetchlings (*Lathyrus* spp.) of the Fabaceae family contain β-cyanoalanine (together with γ-glutamyl-β-cyanoalanine) at high concentrations. The seeds of common vetch (*Vicia sativa*) contain up to 1.5 g/kg β-cyanoalanine and 0.6 g/kg of the γ-glutamyl derivative. Decarboxylation of β-cyanoalanine and other reactions produce β-aminopropionitrile, which is considered to be a cause of the disease lathyrism (see Section 10.3.2.7.1).

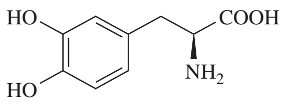
2.2.1.2.5 Aromatic amino acids

Most non-protein aromatic amino acids are derived from L-phenylglycine (**2-47**), L-phenylalanine (**2-4**) or L-tyrosine (**2-4**). For example, various plants contain 3-carboxyphenylalanine, which is accompanied by 3-carboxyphenyltyrosine. Legumes of the genus *Vigna* (Fabaceae) contain 4-aminophenylalanine, the β-phenyl-β-alanine isomer of phenylalanine (**2-47**) occurs in different types of beans (*Phaseolus* spp.).



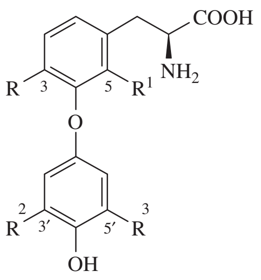
2-47, phenylglycine (*n* = 0)
β-phenyl-β-alanine (*n* = 1)

An important aromatic hydroxyamino acid is L-3,4-dihydroxyphenylalanine, known by the acronym DOPA (from the name **di**hydr**o**xy**p**henyl**a**lanine, **2-48**). It is formed by enzymatic oxidation of tyrosine and becomes the precursor of brown and black pigments (pigments of the eyes, hair, skin or fur of animals) termed melanins (see Section 9.3.1.1). Melanins are formed from DOPA by enzymatic browning reactions that proceed *in vivo*. Clams have this amino acid in special viscous proteins that allow adhesion onto different surfaces.



2-48, 3,4-dihydroxyphenylalanine (DOPA)

Tyrosine is additionally a precursor of the thyroid hormones 3,5,3′,5′-tetraiodothyronine, also known as thyroxine (**2-49**), and 3,5,3′-triiodothyronine, which was previously called iodogorgic acid.



2-49, thyronine, $R = R^1 = R^2 = R^3 = H$
triiodothyronine, $R = R^1 = R^2 = I$, $R^3 = H$
tetraiodothyronine (thyroxine), $R = R^1 = R^2 = R^3 = I$

2.2.1.2.6 Heterocyclic amino acids

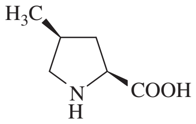
Foods of plant and animal origin often contain amino acids structurally related to proline, the lower and higher homologues and amino acids being derived from histidine and other nitrogen heterocyclic compounds. These amino acids are mainly derivatives of azetidine, pyrazole, pyridine, piperidine, pyrimidine, purine, isoxazole, isoxazoline and other nitrogen heterocycles. Less frequently their ring is derived from oxygen heterocycles (such as pyran-2-ones) or sulfur heterocycles (thiazanes). The side chains are hypothetically derived from glycine, alanine and 2-aminobutyric acid, although the heterocyclic amino acid skeletons are synthesised from other biochemical precursors. Many heterocyclic amino acids have significant biological effects. They often exhibit neurotoxicity, hallucinogenic, insecticidal and other toxic effects.

2.2.1.2.7 Dervivatives of proline, histidine, lysine and cysteine

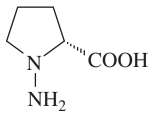
Examples of proline derivatives, which reportedly occur in apples and loquat (Japanese plum; *Eriobotrya japonica*, Rosaceae), are (*S*)-4-methyl-L-proline (**2-50**), (*S*)-4-hydroxymethyl-L-proline and 4-methylene-L-proline. *N*-Amino-D-proline, or linatin (**2-51**), together with the corresponding dipeptide (γ-glutamyl derivative), occurs in linseed (*Linum usitatissimum*, Linaceae). It has antinutritional effects as it acts as an antivitamin B_6. Cucurbitin, (*R*)-3-aminopyrrolidine-3-carboxylic acid (**2-52**), is an unusual compound that is found in the seeds of various squashes. It is responsible for their antihelmintic (vermifugal) effects, which are destructive to parasitic helminth worms. In *Cucurbita pepo*

[1] β-Cyanoalanine can be transformed into γ-glutamyl-β-cyanoalanine by γ-glutamyl transferase. Other enzymes involved in cyanide catabolism are cyanide hydratase and rhodanase. Cyanide hydratase, found in many fungi, catalyses the hydration of cyanide to formamide ($H_2N{-}CH{=}O$). Rhodanase found in bacteria, plants and animals catalyses the conversion of cyanide into thiocyanate ($R{-}S{-}C{\equiv}N$), which is also known as rhodanide.

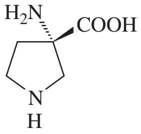
(Cucurbitaceae) squash seeds the concentration ranges from 1.7 to 6.6%, and in *C. maxima* squash seeds it can reach up to 19.4%.



2-50, 4-methylproline

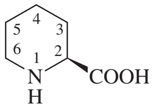


2-51, linatin



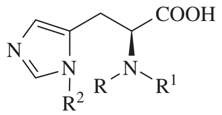
2-52, cucurbitin

The higher homologue of proline, L-pipecolic (L-2-piperidinecarboxylic) acid (**2-53**), is commonly found in many plants. It is a metabolite of L-lysine, which arises from the oxidative deamination of its α-amino group. Pipecolic acid is found at high concentrations in beans (17–44 mg/kg) and cruciferous vegetables (about 12–21 mg/kg). Lower concentrations are found in potatoes (2.5 mg/kg) and fruits (1.3 mg/kg). Some legumes, such as those of the genus *Inga* (originating in tropical America), contain 4-hydroxy-, 5-hydroxy- and 4,5-dihydroxypipecolic acids. Pipecolic acid is also found in animal tissues. In the mammalian brain it acts as a stimulator of γ-aminobutyric acid (**2-11**) receptors. Abnormally high amounts of pipecolic acid in blood plasma (known as hyperpipecolataemia or hyperpipecolic acidaemia) are found in chronic liver diseases and some genetic disorders.

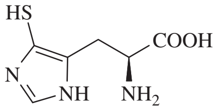


2-53, pipecolic acid

In addition to the previously mentioned 3-methylhistidine derived from 3*H*-imidazole isomer (see Section 2.2.1.1.2), the imidazole skeleton occurs in many other amino acid molecules. The so-called acetylhistidine, diacetylhistidine and triacetylhistidine (**2-54**) are present as free amino acids in spinach (*Spinacia oleracea*, Asteraceae). The free amino acid *N*[1]-methyl-4-mercaptohistidine, known as ovothiol A (**2-55**), can be found in many marine animals. It is one of three mercaptohistidines isolated from the eggs and ovaries of marine invertebrates, where it occurs at high concentrations. Thanks to the imidazole ring, ovothiol A shows a higher reactivity of the thiol group (the active form is thiolate) than glutathione towards free radicals, including reactive oxygen species. In polychaetes (such as *Platynereis dumerilii*) it has the function of a male pheromone. An effective antioxidant is also a 2-thioimidazole betaine known as L-ergothioneine (**2-16**), which is synthesised in Actinomycetales bacteria (such as *Mycobacterium* ssp.) and in various non-yeast-like fungi, including edible genera of the Basidiomycota division, such as *Boletus* ssp. In contrast, no ergothioneine synthesis occurs in higher plants or any animal species. It has been suggested that the incorporation of ergothioneine in plants may result from the absorption of the ergothioneine produced by microorganisms in the soil and in animals through ergothioneine-containing plant and animal foods. Allegedly, King Bolete (*Boletus edulis*) has the highest amount of this amino acid (528 mg/kg fresh weight). Concentrations in other fungi are lower, for instance in oyster mushroom (*Pleurotus ostreatus*, 119 mg/kg), button mushrooms (*Agaricus bisporus*, 0.46 mg/kg), chanterelle (*Cantharellus cibarius*, 0.06 mg/kg fresh weight) and undetectable amounts occur in shiitake (*Lentinula edodes*). Concentrations of ergothioneine in pork liver were 8.71 mg/kg, in pork meat (loin) 1.33 mg/kg, in ham 1.12 mg/kg, in whole grain wheat bread 0.53 mg/kg and in broccoli 0.24 mg/kg.

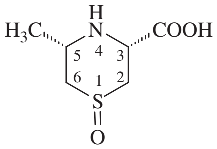


2-54, acetylhistidine, R = $COCH_3$, $R^1 = R^2 = H$
diacetylhistidine, R = $R^1 = COCH_3$, $R^2 = H$
triacetylhistidine, R = $R^1 = R^2 = COCH_3$



2-55, ovothiol A

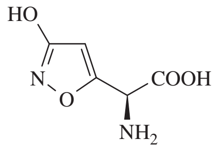
A special sulfur containing heterocyclic amino acid is cycloalliin, (1*S*,3*R*,5*S*)-3-carboxy-5-methyl-1,4-thiazan-*S*-oxide (**2-56**), which is formed in onion and garlic from the amino acid isoalliin (**2-30**). The cycloalliin content in raw onion bulbs and garlic cloves ranges from 0.5 to 1.5 g/kg.



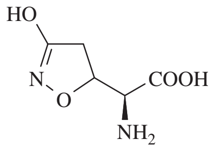
2-56, cycloalliin

2.2.1.2.8 Substituted glycines

Amanitas, such as fly agaric (*Amanita muscaria*), contain a toxic derivative of isoxazole known as ibotenic acid (**2-57**), which is biosynthesised from glutamic acid. Hallucinogenic effects also shows its dihydroderivative tricholomic (dihydroibotenic) acid (**2-58**), which is a component of *Tricholoma muscarium* mushrooms (see Section 10.3.2.9.3).



2-57, ibotenic acid



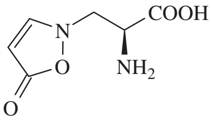
2-58, tricholomic acid

2.2.1.2.9 Substituted alanines

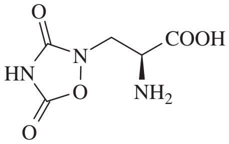
There is a relatively large group of substituted alanines derived from isoxazoline, isoxazolidine and oxazoline. They can be found mainly in germinating seeds and in higher fungi. The donor of the alanyl residue is usually *O*-acetylserine.

An example of these compounds is β-(isoxazolin-5-on-2-yl)-L-alanine also known as 2-(2-amino-2-carboxyethyl)isoxazolin-5-one (**2-59**) which occurs in the seeds and seedlings of many legumes (Fabaceae), vetchlings (*Lathyrus* spp.), vetches (*Vicia* spp.), garden peas (*Pisum* spp.) and lentils (*Lens* spp.). For example, the

amount of β-(isoxazolin-5-on-2-yl)-L-alanine in the seedlings of *L. odoratus* is 0.7–0.8 g/kg fresh weight, in seedlings of lentils 0.17 g/kg and in seedlings of garden peas 1.31 g/kg. Seedlings of lentils also contain a γ-glutamyl derivative of β-(isoxazolin-5-on-2-yl)-L-alanine (0.40 g/kg). β-(Isoxazolin-5-on-2-yl)-L-alanine shows antimycotic effects and, as a structural analogue of glutamic acid (an antagonist), has a neuroexcitatory effect. In vetchlings and vetches, but not in sweet peas, garden peas and lentils, β-(isoxazolin-5-on-2-yl)-L-alanine is a metabolic precursor of 3-aminopropionitrile, L-2,4-diaminobutanoic acid and neurotoxic 3-(*N*-oxalyl)-L-2,3-diaminopropionic acid (β-*N*-oxalyl-L-alanine), which occur in some species of these plants and are responsible for the crippling human disease lathyrism (see Section 10.3.2.7.1). The seeds of the vine *Quisqualis indica* (Combretaceae), known as Rangoon Creeper and native to tropical Asia, contain L-quisqualic acid (**2-60**), which is a stimulant of glutamic acid receptors and has neuroexcitatory activity. Oil extracted from these seeds is used in traditional Chinese medicine as a nematocide and in the treatment of ulcers of the stomach and duodenum. Its leaves, shoots and pods are popular vegetable plants.

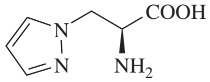


2-59, 2-(2-amino-2-carboxyethyl) isoxazolin-5-one



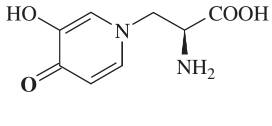
2-60, quisqualic acid

β-Pyrazol-1-yl-L-alanine (**2-61**), derived from pyrazole, occurs in some plants of the *Cucurbitaceae* family. It was first isolated from the seeds of watermelon (*Citrullus lanatus*), where it represents approximately 0.1% of dry matter. It also occurs in the seeds of many other melons, squashes and gourds, together with other heterocyclic amino acids such as 3-aminopyrrolidine-3-carboxylic acid (**2-52**).



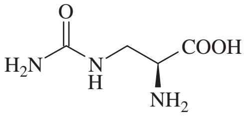
2-61, β-pyrazol-1-ylalanine

The pyridine (dihydropyridine) derived amino acid β-(3-hydroxy-4-pyridon-1-yl)-L-alanine, L-mimosine (**2-62**), is thyreotoxic (causes over-activity of the thyroid gland) to non-ruminant animals. It occurs in the subfamily Mimosoideae of the legume family Fabaceae, in plants of the genera *Mimosa* and *Leucaena*. *Leucaena leucocephala* (syn. *Mimosa glauca*) is native to the tropical and subtropical Americas, known as White Leadtree, and is used as livestock fodder. The mimosine content in seeds can reach up to 5% of dry matter (see Section 10.3.2.7.1). The amino acid pyridosine arising from lysine in the Maillard reaction can also be considered a dihydropyridine derivative (see Section 4.7.5.12.3).



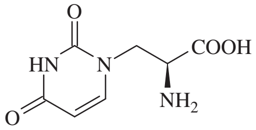
2-62, mimosine

Several other free amino acids derived from purines and pyrimidines have roles as defense agents. Plants of the genus *Mimosa* synthesise 5-aminouracil, which blocks mitotic division and the incorporation of guanosine into nucleic acids. The product of its catabolism is the amino acid L-albizziine (**2-63**), the precursor of L-2,3-diaminopropionic acid (**2-41**) that accumulates in plants.

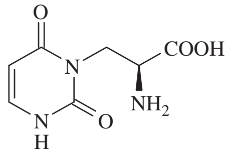


2-63, albizziine

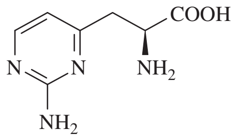
The neuroactive pyrimidine derivative L-willardiine, β-(uracil-1-yl)-L-alanine (**2-64**), occurs in peas (*Pisum sativum*, Fabaceae), the seeds of European beech (*Fagus sylvatica*, Fagaceae) and together with mimosine is present in some acacia (*Acacia* spp., Fabaceae). In subtropical legumes of the genus *Crotalaria* (young pods and leaves of certain species are used as vegetables, others are used as fodder and green manure), it is accompanied by L-isowillardiine, β-(uracil-3-yl)-L-alanine (**2-65**). Amounts of isowillardine in garden pea seedlings, for example, have been reported to be 0.67 g/kg. The amino acid L-lathyrine (**2-66**) occurs in various types of vetchlings (*Lathyrus* spp., Fabaceae). For example, lathyrine makes up 2% of dry matter in seeds of Tangier peas (*Lathyrus tingitanus*). Unlike other *N*-heterocyclic β-substituted alanines, the alanyl residue is derived from L-alanine and not from *O*-acetyl-L-serine.



2-64, willardiine

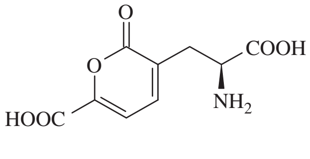


2-65, isowillardiine

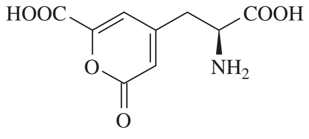


2-66, lathyrine

The fly agaric (*Amanita muscaria*), other Amanitas and some higher plants contain stizolobic acid (L-2-amino-6-carboxy-2-oxo-2*H*-pyrane-4-propionic acid, **2-67**) and stizolobinic acid (L-2-amino-6-carboxy-2-oxo-2*H*-pyrane-3-propionic acid, **2-68**), with DOPA as the precursor. Stizolobic acid is a precursor of the orange betaxanthine muscaaurine II, which is, along with other pigments (see Section 9.3.1.3.1), responsible for the orange–red colour of the hat of this toadstool.



2-67, stizolobic acid

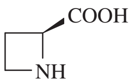


2-68, stizolobinic acid

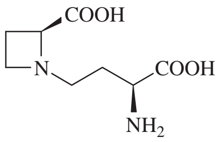
2.2.1.2.10 Substituted aminobutanoic acids

Higher homologues of heterocyclic β-substituted alanines, with another carbon atom in their side chain, can be considered as compounds derived from homoserine or as γ-substituted 2-aminobutanoic acids.

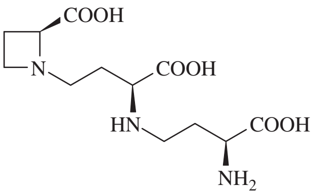
The lower homologue of proline, azetidine-2-carboxylic acid (**2-69**), has antimicrobial effects associated with its incorporation into proteins in place of proline. A small amount of this amino acid is found in some legumes (Fabaceae), plants of the lily family (Lilliaceae), Amaryllidaceae family and red algae (Rhodophycae). Its derivatives with the *N*-alanyl residue, *N*-(3-amino-3-carboxypropyl)azetidine-2-carboxylic acid (**2-70**) and nicotianamine (**2-71**), occur in beechnuts (*Fagus sylvatica*, Fagaceae). Nicotianamine also occurs in Virginia tobacco leaves (*Nicotiana tabacum*, Solanaceae), and the hydroxylated analogue of nicotianamine, mugineic acid (**2-72**), occurs in rice. Tobacco also contains the pyridine derivative L-nicotianine (**2-73**). *N*-(3-Amino-3-carboxypropyl) azetidine-2-carboxylic acid, nicotianamine, mugineic acid and other compounds with similar structures act as phytosiderofores (i.e. they form complexes with iron ions), which allow plants to take in iron from the soil (see Section 6.2.2.1).



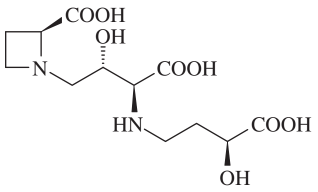
2-69, azetidine-2-carboxylic acid



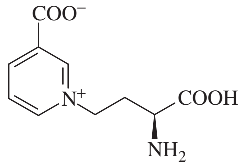
2-70, *N*-(3-amino-3-carboxypropyl)azetidine-2-carboxylic acid



2-71, nicotianamine

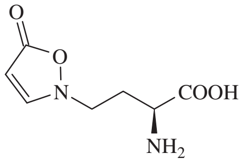


2-72, mugineic acid



2-73, nicotianine

A number of heterocyclic substituted aminobutanoic acids occur in legumes. The sweet pea (*Lathyrus odoratus*) contains 3.5% (dry matter) β-(isoxazolin-5-on-2-yl)alanine, a higher homologue also known as α-amino-γ-(isoxazolin-5-on-1-yl)butanoic acid or 2-(3-amino-3-carboxypropyl)isoxazolin-5-one (**2-74**). This amino acid is neurotoxic. Poisoning shows similar symptoms to poisoning by neurolathyrogenic L-2,4-diaminobutyric acid (see Section 10.3.2.7.1). Other toxic amino acids with a 3-amino-3-carboxypropyl residue are canavanine and canaline (see Section 10.3.2.7.1).



2-74, 2-(3-amino-3-carboxypropyl)isoxazolin-5-one

2.2.2 Physiology and nutrition

All tissues have some capability to biosynthesise the non-essential amino acids, to modify amino acids and to convert non-amino acid carbon skeletons into amino acids. At times of dietary surplus, the potentially toxic nitrogen of amino acids is eliminated via transamination, deamination and urea formation to yield various compounds, which are then used for the biosynthesis of glucose or fatty acids or they may be converted into carbon dioxide and water in the citric acid cycle, which releases energy during times of starvation. Transamination is a process catalysed by amino transferases during which the amino group is transformed into the oxo acid, yielding the oxo acid of the original amino acid and a new amino acid. During oxidative deamination, an amino acid is converted into the corresponding oxo acid by the removal of the amine functional group as ammonia. There are two major pathways for ammonia detoxification by the liver: urea and glutamine synthesis. Approximately 90% of surplus nitrogen in humans enters the urea cycle for irreversible conversion to urea, which is excreted by the kidneys. The major pathway for ammonia fixation in liver is glutamine synthesis. According to the degradation reactions, amino acids are divided into three categories:

- **glucogenic**
- **ketogenic**
- **glucogenic and ketogenic**.

Glucogenic amino acids degrade to pyruvic acid or citric acid cycle intermediates, such as 2-oxoglutaric or oxaloacetic acid, which are precursors of glucose via gluconeogenesis. All amino acids except lysine and leucine are at least partly glucogenic. Lysine and leucine are the only amino acids that are solely ketogenic, giving rise to only acetyl-CoA or acetoacetyl-CoA, which are utilised in the biosynthesis of saturated fatty acids *de novo*. A small group of amino acids, comprising isoleucine, phenylalanine, threonine, tryptophan and tyrosine, give rise to both glucose and fatty acid precursors. Alanine, serine, cysteine and asparagine are converted into oxaloacetic acid. Glutamine, proline, arginine and histidine are converted into 2-oxoglutaric acid via glutamic acid. Succinyl-CoA is a point of entry for non-polar amino acids such as methionine,

valine and isoleucine. Leucine is degraded to acetyl-CoA and acetoacetic acid. Tryptophan, lysine, leucine, phenylalanine, tyrosine and isoleucine donate their carbons to acetyl-CoA.

People with a sufficiently varied and balanced diet (whose energy intake from protein is in the range of 10–15% of their total energy intake and where the ratio of animal and vegetable proteins is in the ratio 1 : 1) usually have a good supply of essential amino acids. Complete proteins contain a balanced set of essential amino acids ideal for humans. Some countries allow enrichment of foods with essential amino acids, especially with limiting amino acids: lysine is usually the limiting amino acid in cereals and **vegetable proteins**; methionine, possibly with cysteine, due to a low content is limiting in beans, meat and dairy proteins; threonine is limiting due to the low content in wheat and rye proteins; and tryptophan, which occurs in low amounts in milk caseins and maize and rice proteins. For example, in Japan and other East Asian countries, lysine and threonine are used to fortify rice; lysine is used for bread fortification and methionine is added to soy products. Problems with the intake of essential amino acids may occur in some unconventional foods, where significant restrictions may lead to a rather monotonous diet. This applies, for example, to vegans and those on macrobiotic diets. These types of diet are inappropriate for children, because the lack of some essential amino acids can lead to serious developmental defects.

The essential amino acids content of livestock feed should be monitored, as the lack of limiting amino acids can significantly decrease the feed consumption and cause weight loss. Essential amino acids are commonly used as feed additives at levels of 0.05–0.2%. Such enhancements are required if plant proteins are to maintain their usage level in animal feeding applications.

Certain risks to human nutrition, and especially to livestock nutrition, may be posed by some toxic amino acids (see Section 10.3.2.7 and Section 10.3.2.9.3).

2.2.3 Properties

2.2.3.1 Acid-base properties

The common amino acids are weak polyprotic acids. The ionisable groups do not show a strong tendency to dissociate, and the degree of dissociation thus depends on the pH of the medium. In the physiological range of pH values, the α-carboxyl groups of amino acids are usually fully dissociated, and the α-amino groups are protonated. Compounds with these properties are called **amphoteric** or **ampholytes** (amphoteric electrolytes). Ampholytes form inner salts, that is dipolar, ambiguous or amphoteric ions called amphions (the German equivalent is zwitterion). An amphion carries a positive or a negative electrical charge (**2-2** and **2-3**), so the resulting electrical charge of the molecule is zero. The predominant forms of almost all amino acids in the physiological environment of animal and plant tissues are amphions. In addition to amphions, in aqueous solutions amino acids may also form (depending on pH) cations (as they react with acids and behave as bases) or anions (when they act as acids, Figure 2.3). Therefore, amino acids are very polar compounds, being fairly soluble in water and in polar solvents; they have high dipole moment values. Also, the melting points (they often melt during decomposition) are high (around 200 °C or higher), therefore they are relatively stable at elevated temperatures.

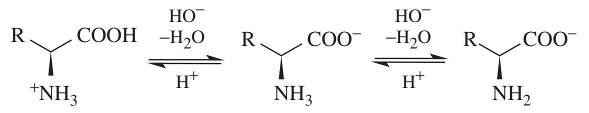


Figure 2.3 Influence of pH on the dissociation of L-amino acids.

2.2.3.1.1 Glycine

The dissociation of the simplest monoaminomonocarboxylic acid glycine as a dependence on the pH is shown schematically in Figure 2.4. In an acidic solution (pH about 2 and below), the predominant form is the ion I_1 (cation) and the net amino acid charge is +1. In a neutral solution (pH value around 6), the ion I_2 (amphion) dominates and the net charge is zero. In alkaline medium (pH approximately 10 and higher), glycine is predominantly present as an I_3 ion (anion) and the net charge of the molecule is −1.

The measure of acidity and basicity of functional groups of amino acids are the **dissociation constants**. The dissociation constant K_1 describes the acidity of the α-carboxyl group of glycine, as shown by the following reaction:

$$^{+}NH_3CH_2COOH + H_2O = {}^{+}NH_3CH_2COO^- + H_3O^+$$

It is therefore defined by the equation (the activities, concentrations of ions and neutral molecules, respectively, are given in parentheses):

$$K_1 = \frac{[^{+}NH_3CH_2COO^-][H_3O^+]}{[^{+}NH_3CH_2COOH][H_2O]}$$

The dissociation constant K_2 describes the basicity of the α-amino group, that is:

$$^{+}NH_3CH_2COO^- + H_2O = NH_2CH_2COO^- + H_3O^+$$

and is defined by the equation:

$$K_2 = \frac{[NH_2CH_2COO^-][H_3O^+]}{[^{+}NH_3CH_2COO^-][H_2O]}$$

The values of the dissociation constants of glycine at 25 °C are: $K_1 = 4.47 \times 10^{-3}$, $K_2 = 1.67 \times 10^{-10}$. Most often these values are shown as negative logarithms, that is as pK_i (−log K_i) values. Then pK_1 = 2.35 and pK_2 = 9.78.

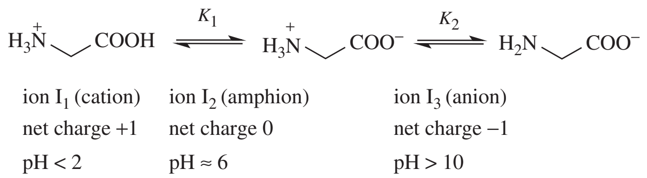


Figure 2.4 Influence of pH on the dissociation of glycine.

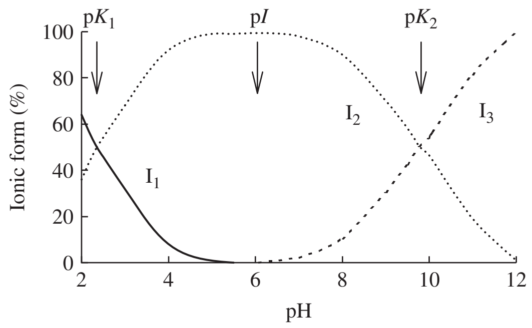


Figure 2.5 Dependence of ionic forms of glycine on pH (see Figure 2.4): — = cation (I_1), - - - - - = amphion (I_2), ········· = anion (I_3).

The pH of the solution at which the maximum concentration of amphions is reached is called the isoelectric point (denoted pI). The value of pI may be calculated as the arithmetic mean of the pK_1 and pK_2 values. The pI value for glycine is:

$$pI = \frac{pK_1 + pK_2}{2} = 6.1$$

At pH = pK_1 the concentrations of the cation and the amphion are equal, and so each of these two forms amounts to 50% of the total amino acid. At pH = pK_2 the concentrations of the amphion and the anion are equal. The dependence of ionic forms of glycine on pH is shown in Figure 2.5.

2.2.3.1.2 Acidic amino acids

A somewhat different situation to that of glycine is found with aspartic and glutamic acids. Owing to the presence of two carboxyl groups in the molecule, these amino acids form four types of ions: cations, amphions and two types of anions. Dissociation of glutamic acid, depending on the pH value of the solution, is given as an example in Figure 2.6. The dependence of the ionic forms of glutamic acid on pH is shown in Figure 2.7.

The amphion again has charge on the α-amino group and on the α-carboxyl, which is more acidic than the distal carboxyl. The value pI (the mean of the pK_1 and pK_3 values) is 3.1. At pH = pI, unlike glycine, the amphion I_2 is not the only ion in solution. It is about 80% of the total amount of glutamic acid present. Cation I_1 is also present at a level of about 10% (this is the main ion in an acidic medium at pH < pK_1), and anion I_3 occurs in the same level. In a neutral solution at pH 7, virtually the only ion present is anion I_3. This ion is a sensory active form of glutamic acid, and is responsible for its unique organoleptic properties. The anion I_3 is the dominant form of glutamic acid in solutions at pH values ranging from pK_2 to pK_3 (pH 4.3–9.5), which in foods is a pH range to which sodium hydrogenglutamate is added as a flavour enhancer (see Section 11.3.5). At pH values around 10 and above, the predominant form of glutamic acid is the anion I_4, which has a net charge of −2. Both carboxyl groups are then dissociated, but dissociation of the amino groups does not normally occur. The other three theoretically possible ionic forms do not actually contribute to the ionic equilibrium. Under suitable conditions (pH and temperature), there is equilibrium between glutamic acid and its lactam L-5-oxopyrrolidine-2-carboxylic acid (see **2-116**). This lactam is in equilibrium with its anion.

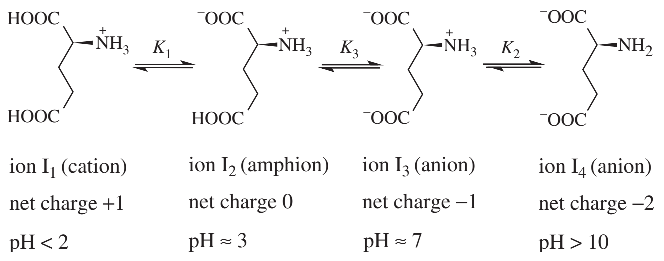


Figure 2.6 Dissociation of glutamic acid as influenced by pH.

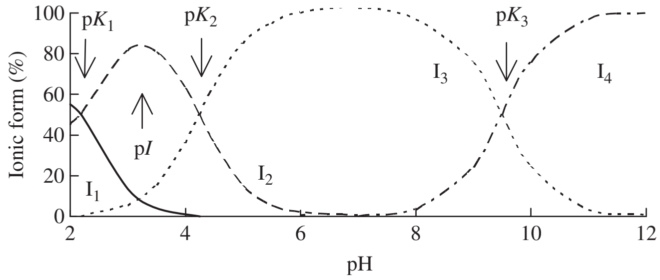


Figure 2.7 Dependence of ionic forms of glutamic acid on pH (see Figure 2.6): — = cation (I_1), - - - - - = amphion (I_2), ········· = anion (I_3), — · — · = anion (I_4).

2.2.3.1.3 Basic amino acids

Just as with glutamic and aspartic acids, lysine also has four types of ions (Figure 2.8). In an acidic solution, two types of cations may occur. At pH < pK_1 (a pH lower than 2), the predominant form is a cation with a net charge of +2, while in the solution around pH 7 the monocation predominates. Its maximum concentration is in solutions of pH equal to the average values of pK_1 and pK_2. The maximum concentration of the amphion is found in solutions of pH around 10, where the pI value is equal to the average values of pK_2 and pK_3. In solutions of pH > pK_3 (pH about 11 and higher), lysine anion predominates (Figure 2.9).

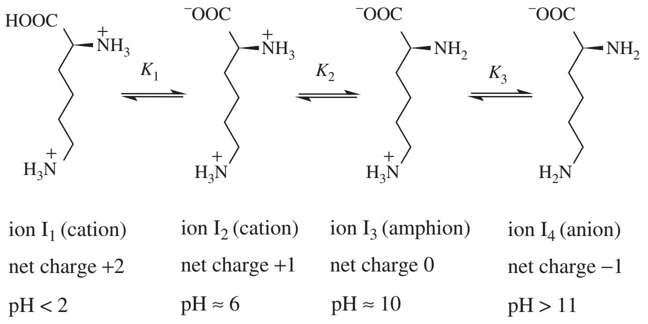


Figure 2.8 Dissociation of lysine as influenced by pH.

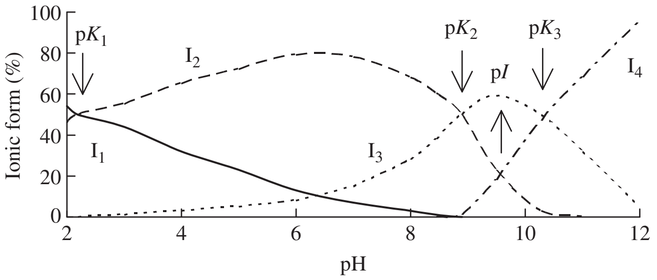


Figure 2.9 Dependence of ionic forms of lysine on pH (see Figure 2.8): — = cation (I_1), - - - - - = cation (I_2), ········· = amphion (I_3), — · — · = anion (I_4).

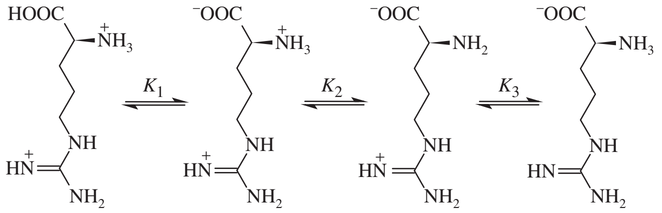


Figure 2.10 Dissociation of arginine as influenced by pH.

As with lysine, four ionic forms can also exist with arginine (Figure 2.10) and histidine (Figure 2.11) depending on the pH. The predominant forms of both amino acids at pH 7 are monocations.

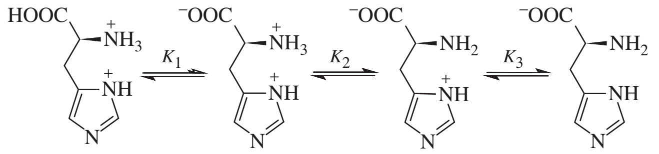


Figure 2.11 Dissociation of histidine as influenced by pH.

2.2.3.1.4 Other amino acids

Other monoaminomonocarboxylic acids behave in an analogous way to glycine. These include alanine, valine, leucine and isoleucine, and include amino acids substituted in the side chain (serine, threonine, methionine, phenylalanine and proline). The cysteine SH-group can also be dissociated at higher pH; therefore, there are four ionic forms. In solutions of pH 7, the main form is the amphion (Figure 2.12), which prevails in tyrosine solutions at pH 7 (analogous to phenylalanine). In an alkaline solution, the hydroxyl group of tyrosine can be deprotonated, forming a phenoxide anion (Figure 2.13). An overview of the pK_i and pI values of proteinogenic and some other common amino acids is given in Table 2.4.

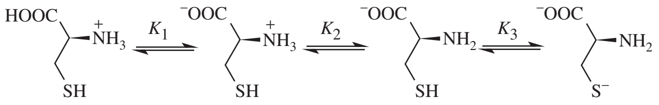


Figure 2.12 Dissociation of cysteine as influenced by pH.

Table 2.4 p*K* and p*I* values of amino acids.

Amino acid	pK_1	pK_2	pK_3[a]	pI	Amino acid	pK_1	pK_2	pK_3[a]	pI
Proteinogenic					Asparagine	2.10	8.84	-	5.5
Glycine	2.34	9.78	-	6.1	Glutamine	2.17	9.13	-	5.7
Alanine	2.35	9.87	-	6.0	Lysine	2.16	9.18	10.79	10.0
Valine	2.29	9.74	-	6.0	Arginine	1.82	8.99	12.48	10.9
Leucine	2.33	9.74	-	6.0	Histidine	1.80	9.33	6.04	7.7
Isoleucine	2.32	9.76	-	6.0	Phenylalanine	2.16	9.18	-	5.7
Serine	2.19	9.21	-	5.7	Tyrosine	2.20	9.11	10.13	5.7
Threonine	2.09	9.10	-	5.6	Tryptofan	2.43	9.44	-	5.9
Cysteine	1.92	10.78	8.33	5.1	Proline	1.99	10.60	-	6.3
Selenocysteine	1.27	8.42	5.24		***Other***				
Methionine	2.28	9.21	-	5.7	4-Hydroxyproline	1.82	9.65	-	5.7
Aspartic acid	1.99	9.90	3.90	3.0	β-Alanine	3.55	10.24	-	6.9
Glutamic acid	2.10	9.47	4.07	3.1	γ-Aminobutyric acid	4.03	10.56	-	7.3

[a] The dissociation constant pK_3 of cysteine (selenocysteine) is the dissociation constant of the –SH (–SeH) group. The dissociation constants of both carboxyl and amino groups of cysteine have values 1.04 and 2.10 and 8.02 and 8.71, respectively. The isoelectric point value is 6.1. The dissociation constant pK_3 of aspartic acid and glutamic acid is the dissociation constant of the distal carboxyl group. The dissociation constant pK_3 of lysine, arginine, histidine and tyrosine is the dissociation constant of the distal amino group (ε-amino group), of the guanidino group, the imino group of imidazole ring and the hydroxyl group, respectively.

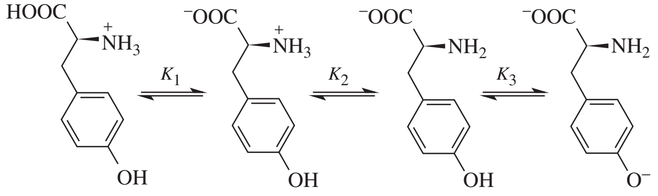


Figure 2.13 Dissociation of tyrosine influenced by pH.

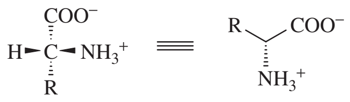
2.2.3.2 Optical activity

With the exception of glycine, all other amino acids contain a chiral carbon atom in the position α to the carboxylic group (**2-2** and **2-3**), which is called a C_α carbon. Therefore each of them exists in two optical isomers (enantiomers). The proteins contain almost exclusively the amino acids of the L-configuration (**2-75**); these amino acids are (*S*)-stereoisomers. The spatial arrangement of substituents around the asymmetric carbon in L-amino acids is the same as in L-glyceraldehyde (see Section 4.2.1.1). The exceptions are L-cysteine and L-selenocysteine that are (*R*)-stereoisomers (the priority of residues $R = CH_2SH$ and $R = CH_2SeH$ is higher than the priority of a carboxyl group COOH). *S*-Substituted cysteine sulfoxides have two chiral centres, at the C_α carbon and the sulfur atom (**2-30**).

2-75, L-amino acid (*S*)-amino acid

Amino acids of the D-series are derived from D-glyceraldehyde and are (*R*)-stereoisomers of amino acids (**2-76**). They are a mirror image of the L-series of amino acids. Compared with L-amino acids, D-amino acids occur relatively rarely in nature. They are mainly bound in a variety of biologically active peptides of plants and animals, occuring in peptidoglycans of microbial cell walls, or as free compounds (such as linatine, **2-51**, in flax seeds). For a long time it was believed that proteins of all living organisms contained only L-amino acids and that D-amino acids were not present in mammals. Recently, D-aspartic acid (synthesised by aspartate racemase) and D-serine (synthesised by serine racemase) were found in proteins of various tissues[2] where they play important roles in living organisms.

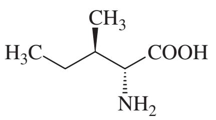
[2] D-Aspartic acid occurs in aorta and lung protein elastin of people with arteriosclerosis, in the protein myelin and peptide β-amyloid of the brains of people with Alzheimer's disease, in the α-crystalline protein in lenses of people with cataracts, in the osteocaleine protein of bones and in the phosphoryne protein of teeth, and D-serine was identified in the β-amyloid of brain of older people as a consequence of aging. D-Aspartic acid and D-serine have important biological functions in brain proteins of living organisms. D-serine functions as a neurotransmitter and modulator, D-aspartic acid plays an important role in the central nervous system and in the regulation of production of hormones in the endocrine glands.



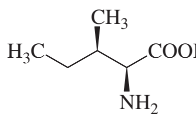
2-76, D-amino acid (*R*)-amino acid

Free D-amino acids found in fresh or heat-processed foods are mostly of microbial origin. They are formed by hydrolysis of peptidoglycans in the cell walls of microorganisms, or by the action of microbial racemases on L-amino acids. Therefore they mainly occur in foods produced by lactic acid fermentation, such as yoghurt and are termed **biogenic** D-amino acids. D-Amino acids may also form through non-enzymatic isomerisation of L-amino acids in foods processed at high temperatures, and especially in foods treated with alkaline reagents. These amino acids are bound in proteins from which they are released by hydrolysis. They are called **abiogenic** D-amino acids.

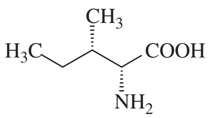
Isoleucine, threonine, 4-hydroxyproline and 3-hydroxyproline also have an asymmetric carbon atom in the side chain (two chiral centres in total). They can therefore occur in four stereoisomeric forms (this number is equal to $2^n = 2^2 = 4$, where $n =$ number of asymmetric carbon atoms) known as L-, D-, L-*allo*- and D-*allo*- according to the configuration of the carbon carrying the amino group. The unique *R*/*S* system is not widely used in biochemistry. The stereoisomers of isoleucine are thus called L-isoleucine or (2*S*,3*S*)-isoleucine (**2-4**), D-isoleucine or (2*R*,3*R*)-isoleucine (**2-77**), L-*allo*-isoleucine or (2*S*,3*R*)-isoleucine (**2-78**) and D-*allo*-isoleucine or (2*R*,3*S*)-isoleucine (**2-79**). Proteins contain only L-isoleucine. The product of enzymatic or thermal isomerisation of L-isoleucine is D-*allo*-isoleucine. Likewise, proteins contain L-threonine or (2*S*,3*R*)-threonine (**2-4**) and L-4-hydroxyproline or (2*S*,4*S*)-4-hydroxyproline (**2-6**). Cystine (as well as lanthionine, see Section 2.5.1.3.4) contains two identical asymmetric carbons in the molecule and, therefore, only exists in three different isomers. Proteins contain just L-cystine (**2-4**), its isomer is D-cystine (**2-80**) and the mesoform *meso*-cystine (**2-81**) is a symmetrical molecule.



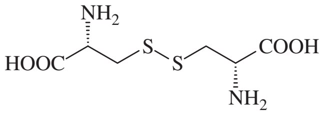
2-77, D-isoleucine



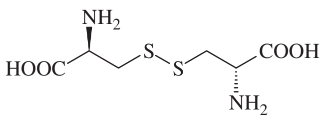
2-78, L-*allo*-isoleucine



2-79, D-*allo*-isoleucine



2-80, D-cystine



2-81, *meso*-cystine

The sorting of amino acids into L- or D- series is not linked to their optical rotation. For example, an aqueous solution of natural alanine or glutamic acid is dextrorotatory, denoted (+)-L-Ala and (+)-L-Glu, respectively. Dextrorotation (turning of polarised light by solutions of certain optically active substances to the right, i.e. clockwise) shows a total of 12 proteinogenic amino acids, while the remaining amino acid shows laevorotation. For example, aqueous solutions of leucine and histidine are laevorotatory, and are denoted (−)-L-Leu and (−)-L-His, respectively. Optical rotation depends greatly on pH. For example, leucine in acidic and alkaline solutions is dextrorotatory; histidine is dextrorotatory in acidic and laevorotatory in neutral and alkaline solutions.

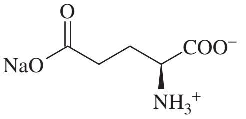
2.2.3.3 Organoleptic properties

Some amino acids are sensorially active substances, which can therefore influence the organoleptic properties of food. For example, glycine has a sweet taste (70% of the sweetness of sucrose) and is sometimes mixed with saccharin as a sweetening agent. According to their organoleptic properties, the proteinogenic amino acids can be classified as:

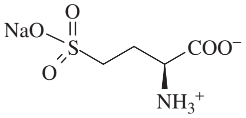
- **sweet** (glycine, alanine, threonine, proline and hydroxyproline)
- **acidic** (aspartic and glutamic acids)
- **bitter** (amino acids with hydrophobic side chains, i.e. valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan)
- **indifferent** (other amino acids).

Free amino acids are only found in small amounts in most foods. Therefore, they only influence the flavour of foods when production is based on proteolysis (some cheeses, but also meat and fish). Enzymatic hydrolysates of proteins (such as soy sauces) or hydrolysed vegetable proteins (acid hydrolysates), which contain only amino acids, are used extensively as seasonings.

Glutamic acid and its monosodium salt, sodium hydrogen L-glutamate (**2-82**), have unique organoleptic properties. It is salty, but also has the taste known as **umami** (see Section 8.3.3.1), therefore it is used as a food additive (to intensify the flavour of meat and vegetable dishes) for the production of various spice products. The salts of some other amino acids have more or less comparable taste, for example salts of L-ibotenic acid (**2-57**), L-tricholomic acid (**2-58**), (*S*)-2-amino-4-sulfobutanoic acid known as L-homocysteic acid (**2-83**), L-theanine (**2-40**), 2-methyl-L-glutamic acid, L-5-oxopyrrolidine-2-carboxylic acid (also known as pyroglutamic acid or glutiminic acid or 5-oxoproline; see **2-116**) and some other compounds.



2-82, sodium hydrogen glutamate



2-83, homocysteic acid sodium salt

2.3 Peptides

Peptides have a number of different functions in organisms and often exhibit significant biological effects. Some are widely distributed in nature and are found in many diverse organisms, whereas others have a restricted occurrence. Many oligopeptides and polypeptides show physiological effects, as they act like hormones (for example, the linear peptides secretin, insulin and thyroliberin and the cyclic peptides oxytocin or vasopressin) and antibiotics (gramicidine, polymyxin, bacitracin and other peptide antibiotics).

Many microbial peptides, collectively known as **bacteriocins**, have antibacterial properties. In practice, one of these peptides, the polycyclic peptide nisin, is used as a food preservative (see Section 11.2.1.2.1). It is produced by the metabolic activity of the bacterium *Lactococcus lactis* and used in dairy technology to stabilise fermented products. Nisin contains five sulfide bridges and 34 amino acid units, including the uncommon amino acids D-alanine, D-2-aminobutyric acid, 2-aminoacrylic acid, known as dehydroalanine (Dha), 2-aminocrotonic acid, or dehydrobutryine (Dhb), 3-methyllanthionine (β-methyllanthionine, MeLan) and *meso*-lanthionine (Lan) cross-links (see Section 2.2.1.2.2). Nisin acts against gram-positive organisms (*Streptococcus* spp. and *Clostridium* spp.) and against the vegetative forms and spores.

Furthermore, peptides include a number of toxins that are highly effective towards microorganisms, such as botulinum toxin produced by the bacteria *Clostridium botulinum* (see Section 12.3.2.1), cyclic toxins of higher fungi, such as fallotoxins and amatoxins of toxic agarics of the genus *Amanita* (see Section 10.3.2.9.2), insect toxins such as apamine and mellitine in bee venom, and toxins of many other insect species (spiders, scorpions, reptiles and amphibians).

Some peptides that are present in foods significantly influence their organoleptic properties, especially the taste.

2.3.1 Structure, nomenclature and classification

Peptides are amino acid polymers in which the carboxyl group of one amino acid bonds to the amino group of the second amino acid via an **amide bond**. The amide bond thus created is called a **peptide bond** because of its specific nature.[3] The combination (condensation) of two α-amino acids yields a dipeptide, three molecules of amino acids produce a tripeptide and so on. The formation of a dipeptide from two amino acids is accompanied by the loss of a water molecule. Each linear peptide contains one amino acid with a free carboxyl group, which is a ***C*-terminal amino acid**, and one amino acid with a free amino group, an ***N*-terminal**

[3]Amide bonds resulting from the condensation of amino groups of α-amino acids with carboxylic groups of organic acids are found in many other compounds but these are not classified as peptides. Examples are some vitamins such as pantothenic acid, tetrahydrofolic acid (folic acid with two or more molecules of glutamic acid are already peptides) and the active form of biotin (biocytin).

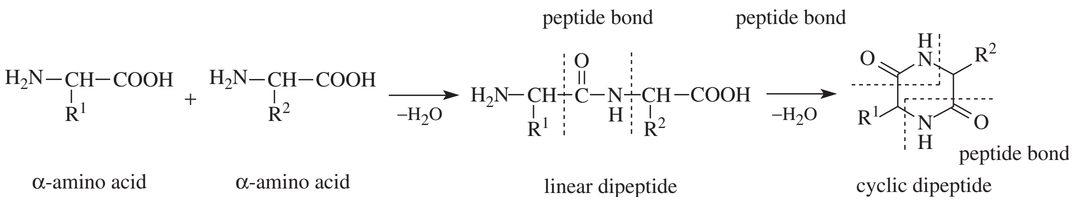


Figure 2.14 Formation of linear and cyclic dipeptides.

amino acid. In addition to linear peptides, the condensation of amino acids also leads to cyclic structures, which have no free carboxyl group and no free amino group. Such compounds are called cyclic peptides. Examples of simple cyclic dipeptides are 3,6-disubstituted 2,5-dioxopiperazines (Figure 2.14).

The peptide bond is essentially a rigid planar structure. In dipeptides, six atoms lie in the same plane: the α-carbon atom and CO group from the first amino acid and the NH group and α-carbon atom from the second amino acid. The peptide bond shows substantial double-bond characteristics, which prevents rotation about this bond (Figure 2.15). Thanks to the rigidity of the bond, peptide groups may be present in *cis* or *trans* conformations (Figure 2.16). The *trans* form is strongly favoured; therefore natural peptides and proteins contain almost exclusively the energetically preferable *trans* conformers, where the neighbouring C_α atoms are situated on opposite sides of the peptide bond. The bond distances between the atoms of the peptide groups of *trans* and *cis* conformers are (in nm): C–N = 0.133 (0.132), C(=O) = 0.124 (0.124), N–H = 0.100 (0.100), NC_α = 0.146 (0.147) and C–C_α = 0.151 (0.153). Typically the *cis* conformation only occurs when the nitrogen atom in the peptide bond is from proline. In the peptide bond region, only substituents on C_α carbons can freely rotate, and thus they determine the conformational structure of the peptide chain. The C_α carbon attachment into the peptide chain involves two single bonds, a C_α–N bond and a C_α–C bond. Other groups of the peptide chain can freely rotate around these bonds. The degree of rotation can

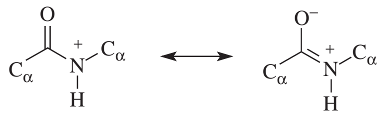


Figure 2.15 Peptide bond (substituents at the C_α are not marked).

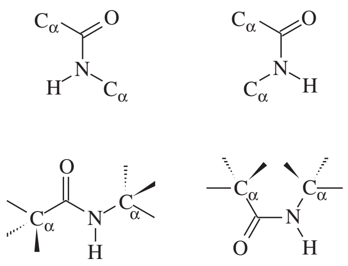


Figure 2.16 Peptide bond *trans* and *cis* conformations.

be specified by dihedral angles of rotation around the bond C_α–N (angle φ; phi) and bond C_α–C (angle ψ; psi), which are sometimes called torsion angles. The different values of these angles give rise to a large number of conformations of peptides and proteins.

Peptides are usually classified according to the molecule size (number of bound amino acids), the shape of the chain and the type of links in the chain, among other things. According to the number of bound amino acids (monomers), peptides are divided into:

- **oligopeptides** (containing typically 2–10 amino acid molecules in the chain)
- **polypeptides** (containing typically 11–100 amino acid molecules or compounds with relative molecular weights of about 10 kDa; polypeptides, formerly known as **macropeptides**, actually produce the proteins).

According to the chain type, peptides are divided into:

- **linear peptides** (which represent the clear majority)
- **cyclic peptides**.

Peptides containing just amino acids (often the less common amino acids) are called **homeomeric peptides**, and peptides containing other compounds are **heteromeric peptides** or **peptoids**. This group includes:

- **nucleopeptides**
- **lipopeptides**
- **glycopeptides**
- **phosphopeptides**
- **chromopeptides**
- **metalopeptides**.

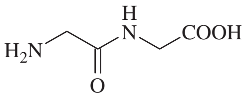
Peptides in which all of the covalent linkages between the constituent amino acids are peptide bonds are termed **homodetic peptides**, whereas those that contain peptide bonds together with other covalent linkages between certain amino acid residues are called **heterodetic peptides**. Examples of these covalent linkages

are disulfide bonds (R–S–S–R′), thioester bonds (R–CO–S–R′) or ester bonds (R–CO–O–R′). The relevant peptides (with one or more ester bonds) are **depsipeptides**. The peptide bonds usually contain bound α-amino groups and α-carboxyl groups. Unlike in proteins, the peptide bonds in peptides sometimes contain the distal functional groups, such as the γ-carboxyl group of glutamic acid in the so-called γ-peptide bond. Unlike proteins, peptides frequently contain D-amino acids and unusual amino acids, for example β-alanine (**2-10**), taurine (see Section 5.15), γ-aminobutyric acid (**2-11**), pyroglutamic acid (see **2-111**) and others.

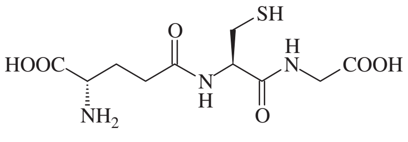
The terminology considers linear peptides as acylated amino acids. Therefore, the amino acid at the carboxyl terminus of the chain is what gives it its name; for the other amino acids, the ending -ine is replaced by the suffix -yl. The practice uses three letters for labelling peptide chains or one letter for symbols for amino acid. The simplest dipeptide composed of two molecules of glycine is called glycylglycine (**2-84**), or for short Gly-Gly or G-G. The important tripeptide with the trivial name of glutathione (**2-85**) is γ-L-glutamyl-L-cysteinylglycine. Using the three letter symbols, glutathione is:

Glu⌊ Cys-Gly or γ Glu⌊ Cys-Gly

using one letter amino acid symbols, glutathione is ECG. The symbol ⌊ indicates the distal carboxyl group linkage. Nomenclature of cyclic peptides is based on either the peptide or the heterocycle. For example, the cyclic dipeptide derived from glycine is cyclo(Gly-Gly) or 2,5-dioxopiperazine (Figure 2.14, R = H). Most naturally occurring cyclic peptides contain a higher number of amino acids.



2-84, glycylglycine



2-85, glutathione

2.3.2 Biochemistry

Peptides are biosynthesised by both ribosomal and non-ribosomal processes from a wide range of amino acids. Ribosomal peptide biosynthesis leads to peptide enzymes, hormones and many other physiologically active substances. Many structures biosynthesised by ribosomal processes are post-translationally modified. Glycopeptides are produced by adding sugar residues via *O*-glycoside linkages to the hydroxyls of serine and threonine residues or via *N*-glycoside linkages to the amino group of asparagine. With phosphopeptides, the hydroxyl groups of serine or threonine are esterified with phosphoric acid. Many peptides contain a pyroglutamic acid residue at the *N*-terminus, which is a consequence of glutamine intramolecular cyclisation between the γ-carboxyl and the α-amino group. The *C*-terminal carboxylic acids may also frequently be converted into an amide. Non-ribosomal processes synthesise many natural peptides that occur in foods. They are responsible for the formation of glutathione, histidine-containing dipeptides of skeletal muscles, peptide toxins and peptide antibiotics. These peptides are formed via a sequence of enzyme-controlled reactions.

2.3.3 Occurrence

Peptides are found in foods as products of metabolism as a result of the genetic dispositions of the animal or plant organisms. In living organisms, peptides arise either from amino acids by simple biosynthesis (in contrast to proteins this is without the use of the proteosynthetic apparatus), or by hydrolysis of precursors produced in protein synthesis (with possible chemical modification). During processing and storage of foods, peptides also form as secondary products through enzymatic and non-enzymatic hydrolysis of proteins. The process is known as **proteolysis**. Peptides can also arise when amino acids are heated to higher temperatures, but this reaction has no significance in practice.

2.3.3.1 Important peptides

2.3.3.1.1 Glutathione

Tripeptide glutathione, γ-L-glutamyl-L-cysteinylglycine (**2-85**), is a common peptide, which is found in animal and plant tissues and microorganisms. It occurs in two forms, a reduced form (denoted G-SH) and an oxidised form (G-S-S-G), which are part of an important redox system, similar to cysteine and cystine:

$$2\text{G-SH} \rightarrow \text{G-S-S-G} + 2\text{H}$$

$$\text{G-S-S-G} \rightarrow 2\text{H} + 2\text{G-SH}$$

Glutathione is the most abundant non-protein thiol in cells and is also a cofactor of some enzymes, such as glyoxylase. In animal cells, glutathione is present in high levels of 300–1500 mg/kg, higher plants contain lower amounts of glutathione (wheat flour 10–15 mg/kg) and microorganisms, such as yeast *Saccharomyces cerevisiae*, about 5 000 mg/kg dry cell weight.

The reactivity of the thiol group makes glutathione particularly suitable for a broad range of biochemical functions in all organisms. The glutathione redox system of reduced and oxidised glutathione has an oxidation reduction potential of –0.23 V, which allows it to act as an effective electron acceptor and donor for numerous chemical and biochemical reactions. Glutathione also modifies protein sulfhydryl groups via a number of reactions: reduction of protein sulfenic acids, formation of protein mixed disulfides and their subsequent reduction. For example, glutathione in flour affects the rheological properties of dough (see Section 5.14.6.1.6). The nucleophilic thiol group of glutathione allows reactions with reactive oxygen and reactive nitrogen radicals, hydrogen peroxide and hydroperoxides of fatty acids, formation of mercaptides with metals and reactions with various electrophiles. Thus glutathione is an ideal substance to protect organisms against oxidative stress, poisoning by metal ions and poisoning by a number of exogenous and endogenous toxic organic substances, including carcinogens.

Oxygen and nitrogen radical species are removed from organisms via non-enzymatic reduction with G-SH, whereas the removal of

hydrogen peroxide and hydroperoxides requires enzymatic catalysis by glutathione peroxidases. Glutathione peroxidases (GPx) are antioxidant selenoenzymes, which protect various organisms from oxidative stresses by catalysing the reduction of hydrogen peroxide and fatty acid hydroperoxides at the expense of G-SH (see Section 6.3.15.1.2). Both reactions ensure removal of lipid hydroperoxides from damaged biological membranes (lipoproteins) and lead to the generation of glutathione disulfide (G-S-S-G), which is reduced back to G-SH through the ascorbate/G-SH cycle in the plastids of plants and in erythrocytes of animals by glutathione reductase that uses NADPH from the pentose phosphate shunt. In plants, glutathione is also essential for the protection of membranes by maintaining α tocopherol and zeaxanthin in the reduced state through reaction with ascorbic acid.

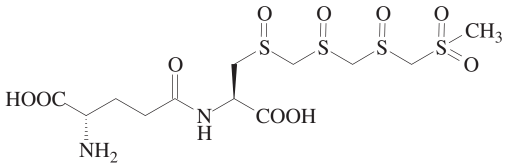
Class III metallothioneins (see Section 6.2.2.2) are metal-binding plant and microbial peptides termed phytochelatins. Phytochelatins are oligomers of glutathione, $(\gamma\text{Glu-Cys})_n$-Gly, which are synthesised by phytochelatin synthase. Phytochelatins are made in the cytosol where they have a high affinity to bind with some metals (e.g. copper and cadmium). These metal–phytochelatin complexes are then transported into vacuoles and the metals are thus sequestered away from sensitive enzymes. In animals, glutathione, either independently or along with nutrients such as zinc or selenium, has an impact on the ability of the body to handle metals such as cadmium, lead, iron and mercury and metalloids such as arsenic, which have the potential to disrupt the metabolism and biological activities of many proteins, because of their high affinity for free sulfhydryl groups.

Glutathione is also closely linked with immunity, protecting the cells and assisting the liver in detoxifying harmful compounds and toxins. Conjugation of G-SH with electrophilic compounds, mediated by the glutathione *S*-transferases (GSTs) enzymes serves to protect cells from toxic chemicals. In plants, these conjugates are transported into vacuoles. In animals, G-SH conjugates are excreted in the urine and faeces. The formation and excretion of G-SH conjugates leads to G-SH depletion, which is attenuated by *de novo* synthesis of G-SH. If glutathione is depleted, an organism can be predisposed to the effects of stress from xenobiotics. When taking glutathione, vitamin C is also recommended because ascorbic acid assists the glutathione to maintain its powerful free radical-suppressing effects.

2.3.3.1.2 Other glutamyl peptides

Both animal and plant tissues contain many different γ-L-glutamyl peptides that have a number of important functions. For example, plant γ-glutamyl peptides play a role in the transport of amino acids through membranes, they protect plant cells against the effects of phytotoxic heavy metals (by acting as phytochelatins; see Section 6.2.2.2), in *Allium* and *Brassica* plants they have a key role as a reserve for nitrogen, sulfur (contain up to 50% of organically bound sulfur) and selenium compounds and are precursors of the biologically active amino acids *S*-alk(en)ylcysteine sulfoxides. They are not cleaved by the enzyme alliinase and are thus not directly involved in the aroma formation in onion, garlic and other vegetables. γ-Glutamyl peptides are formed in reactions catalysed by the enzyme L-γ-glutamyl transferase, which catalyses the splitting of the γ-glutamyl linkages in reduced glutathione (G-SH) and the transfer of the glutamate moiety to amino acid or peptide acceptors.

Plants of the genus *Allium* typically contain more than 20 γ-glutamyl peptides, such as *N*-(γ-glutamyl)-*S*-alk(en)yl glutathiones, *N*-(γ-glutamyl)-*S*-alk(en)yl cysteines and *N*-(γ-glutamyl)-*S*-alk(en)yl cysteine sulfoxides. These peptides occur as intermediates in the biosynthesis of *S*-alk(en)ylcysteine sulfoxides in *Allium* vegetables (Figure 2.17). The main γ-glutamyl peptides in garlic cloves are *N*-(γ-glutamyl)-*S*-(prop-2-en-1-yl)-L-cysteine ($R{=}CH_2{-}CH{=}CH_2$) and (*E*)-*N*-γ-glutamyl-*S*-(prop-1-en-1-yl)-L-cysteine ($R{=}CH{=}CH_2{-}CH_3$), which are present at levels of approximately 2.9–4.6 g/kg of fresh weight and 4.6–5.4 g/kg of fresh weigh, respectively. They are followed by *N*-(γ-glutamyl)-*S*-(2-carboxypropyl)-L-cysteinyl glycine, $R{=}\ CH_2{-}CH(COOH)CH_3$, known as *S*-(2-carboxypropyl)glutathione (0.8–1.2 g/kg of fresh weight), *N*-(γ-glutamyl)-*S*-methyl-L-cysteine (0.04–0.4 g/kg of fresh weight) and *N*-(γ-glutamyl)-*S*-(prop-2-en-1-yl)-L-cysteine sulfoxide ($R{=}CH_2{-}CH{=}CH_2$; 0.04–0.08 g/kg of fresh weight). *S*-(2-Carboxypropyl)glutathione is the main γ-glutamyl peptide of onion bulbs (0.1–0.9 g/kg of fresh weight), where it is accompanied by (*E*)-*N*-γ-glutamyl-*S*-(prop-1-en-1-yl)-L-cysteine sulfoxide ($R{=}CH{=}CH_2{-}CH_3$; 0.2–2.3 g/kg of fresh weight), (*E*)-*N*-γ-glutamyl-*S*-(prop-1-en-1-yl)-L-cysteine (up to 0.1 g/kg of fresh weight) and *N*-(γ-glutamyl)-*S*-methyl-L-cysteine (up to 0.1 g/kg of dry weight). In pre-bulbing onions, levels of (*E*)-γ-glutamyl-*S*-(prop-1-en-1-yl)cysteine sulfoxide and *S*-(2-carboxypropyl)glutathione were found to be below 0.05 g/kg of fresh weight and at the bulbing onion stage they amounted to 2.1 and 4.0 g/kg of fresh weight, respectively. The main γ-glutamyl peptide in beans is *N*-(γ-L-glutamyl)-*S*-methyl-L-cysteine, and an important γ-glutamyl peptide of shiitake mushrooms (*Lentinula edodes*) is known as lentinic acid (**2-86**; see Section 2.1.1.2.2).



2-86, lentinic acid

2.3.3.1.3 Histidine dipeptides

In addition to glutathione, the muscle tissue of animals contains a group of dipeptides derived from L-histidine (its 1*H*- and 3*H*-isomers, respectively). These dipeptides include carnosine (β-alanylhistidine, **2-87**), anserine (β-alanyl-1-methylhistidine, **2-88**), balenine also known as ofidine (β-alanyl-3-methylhistidine, **2-89**) and homocarnosine (γ-aminobutyrylhistidine, **2-90**). The biological role of these dipeptides is not yet fully known. It is assumed that they are involved in the contraction of skeletal muscles, and are associated with some enzymes containing copper in the molecule that show the activity of neurotransmitters, vasodilators and modulators. Their buffering capacity at physiological pH values is also important (they are present as cations with an ionised primary

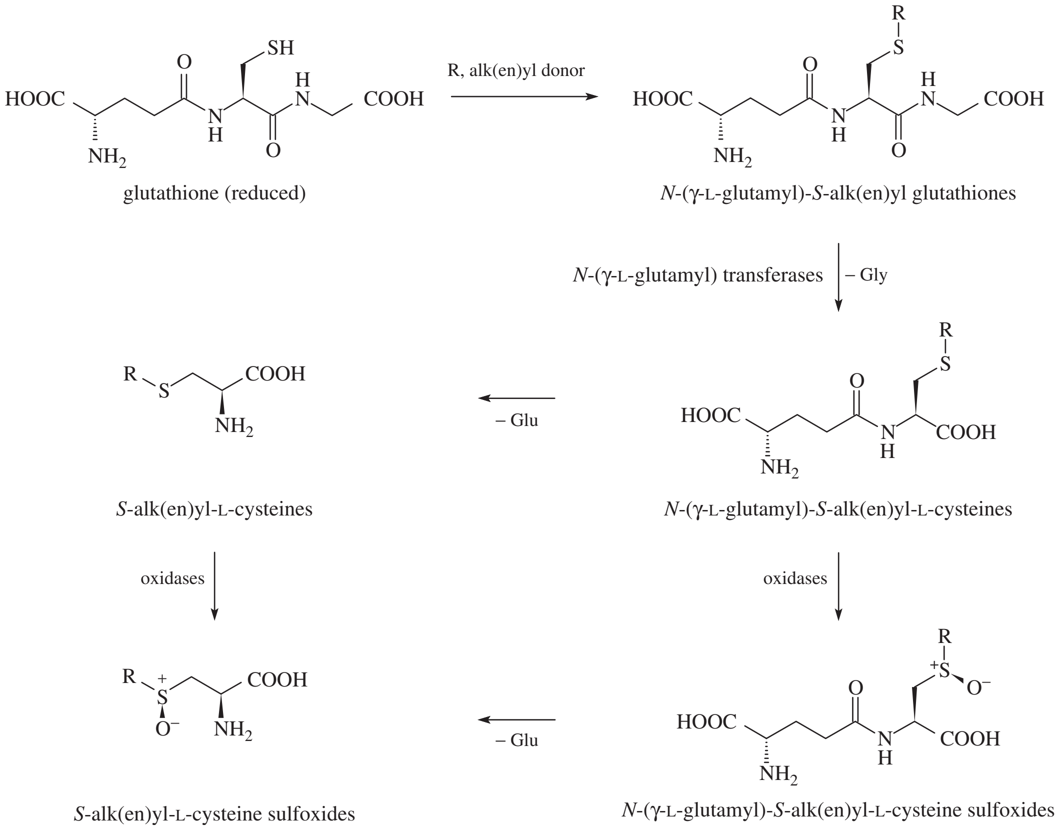
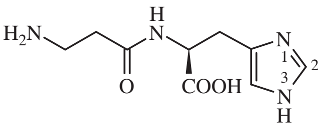
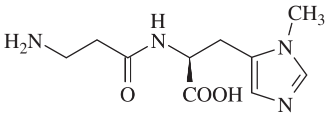


Figure 2.17 Formation of *S*-alk(en)ylcysteine sulfoxides from γ-glutamyl cysteine peptides in *Allium* vegetables.

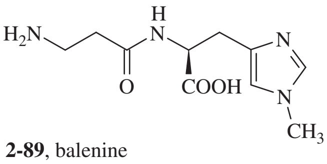
amino group, imidazole ring nitrogen and carboxyl group). Carnosine has recently appeared in some food supplements for athletes, but its use is not yet supported by credible scientific results.



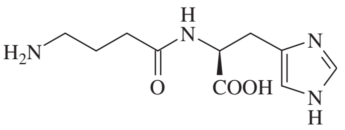
2-87, carnosine



2-88, anserine



2-89, balenine



2-90, homocarnosine

Because of the variations in different types of meat, the dipeptide contents can serve as criteria for determining the origin of meat and meat products (Table 2.5).[4] For example, anserine is the dominant dipeptide in meat from birds, while in animal tissues the main dipeptides are anserine and carnosine (while human tissues contain only carnosine). Determination of these two peptides has been used, for example, to evaluate the amount of chicken meat in pork meat products. Balenine is usually present in small amounts in meat from snakes. In marine mammals such as whales, balenine is the main histidine dipeptide.

The sensory properties of histidine dipeptides and their reaction products may affect the oral sensation of meat products as they contribute to the umami taste (see Section 8.3.3.1) and enhance the flavouring properties of sodium hydrogen glutamate. The key contributors to the typical chicken broth flavour are, for example, anserine, carnosine and β-alanylglycine. During the processing of meat, histidine dipeptides are partly hydrolysed by endogenous peptidases (for example, during curing of ham, about one half of these dipeptides are hydrolysed) and non-enzymatically. They also partly react with protein-bound asparagine and glutamine and are incorporated into proteins during heating. In beef soup stock solution and in model experiments, hydrolysis of proteins with incorporated carnosine yields β-aspartyl-β-alanylhistidine and γ-glutamyl-β-alanylhistidine tripeptides, respectively. During thermal processing of meat, and particularly of fish, histidine dipeptides enter the Maillard reaction (see Section 4.7.5).

[4]The content of individual dipeptides, of course, varies in different muscles and depends on many factors, such as age of the animal and type of processing. Breast muscles of poultry, for example, contain about 5–10 times higher amounts of carnosine and five times higher amounts of anserine than leg muscles (white breast muscles are involved in intense anaerobic metabolism and therefore require a higher buffering capacity).

2.3.3.2 Proteolytic products

Proteolytic enzymes are present in virtually all raw food materials, therefore proteins can be broken down by partial spontaneous

Table 2.5 Histidine dipeptides content of fresh meat.

Meat	Content (mg/kg)			Meat	Content (mg/kg)		
	Carnosine	Anserine	Balenine		Carnosine	Anserine	Balenine
Pork	1 040–4 190	70–160	180	Horse	3 820–4 023	30–48	0
Beef	1 520–3 650	110–552	17	Rabbit	497	4 536	0
Sheep	670–1 898	430–1 992	24	Chicken	100–1 117	550–3 350	-
Goat	520–1 030	750–2 016	0	Turkey	1 600–2 400	6 150	-

hydrolysis during the storage of these materials and during food production, which leads to a mixture of different peptides. In some technologies spontaneous proteolysis is desirable, such as during meat curing and in cheese[5] and malt production.

For example, intense proteolyses of muscle proteins, due to the action of endogenous proteolytic enzymes, have been reported to occur during the processing of dry-cured ham. This gives rise to the formation of free amino acids and short peptides (especially from actin through the action of cathepsin D in meat and from caseines as a result of plasmin and other proteases in cheeses) that contribute directly or indirectly to the flavour characteristics of the final product. In the case of octapeptide Lys-Gly-Asp-Glu-Glu-Ser-Leu-Ala, isolated from beef broth, this reportedly showed umami taste with a threshold value of about 500 mg/l.

Dozens of different peptides have been identified in cheeses. Most of them arise from α_{S1}- and β-caseins and a few are from α_{S2}- and κ-caseins. The proteinases involved in hydrolysis of α_{S1}-casein are mainly cathepsin D originating from milk and cell-envelope proteinase from thermophilic starters, while β- and α_{S2}-caseins are mainly hydrolysed by plasmin. Moreover, peptidases from starters are also active throughout the ripening process, presumably similar to those from non-starter lactic acid bacteria. For example, the bitterness of mature Gouda cheese is caused by calcium and magnesium chlorides, some bitter-tasting free amino acids and is modified by peptides, which arise from the hydrolysis of β-casein (such as decapeptide Tyr-Pro-Phe-Pro-Gly-Pro-Ile-His-Asn-Ser and derived nonanpeptide without the terminal serine) and α_{S1}-casein (tetrapeptide Leu-Pro-Gln-Glu).

Some proteins (especially lipid transfer protein and protein Z, see Section 2.4.5.4.1) and hydrophobic polypeptides resulting from barley proteins in malt, through endogenous proteases and yeast autolysis, are important foam stabilisers in beer.

2.3.3.2.1 Protein hydrolysates and pyrolysates

Linear peptides

Many food products (including soy sauce and other oriental sauces) that are produced by enzymatic hydrolysis of proteins or protein-rich materials contain various peptides. Other materials containing variable amounts of peptides include yeast autolysates and also blood, whey and casein hydrolysates, which are used as food additives of high nutritional value.

During the partial enzymatic hydrolysis of proteins, bitter-tasting peptides are often released, which limits the application of these hydrolysates in food processing. Casein and soy protein hydrolysates in particular have a tendency towards bitterness. The bitter taste is caused by peptides containing hydrophobic amino acids (valine, leucine, isoleucine, phenylalanine, tyrosine and tryptophan). Proline (which commonly occurs in the penultimate position) and pyroglutamic acid are also often present in bitter peptides, while cysteine and methionine are usually absent. Bitter peptides contain varying numbers of bound amino acids, usually between 2 and 23. Several tens of bitter peptides have been characterised. Those isolated from Cheddar cheese, originating from α_{S1}-casein, have the following sequences of amino acids: Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln, Leu-Pro-Gln-Glu, Arg-Pro-Lys-His-Pro-Ile-Lys and Lys-Pro-Trp-Ile-Gln-Pro-Lys. The bitter peptide of Cheddar cheese originating from β-casein is the linear nonapeptide Val-Pro-Gly-Glu-Ile-Val-Glu-Ser-Leu. Another example of a bitter peptide occurring in β-casein hydrolysate is the linear pentadecapeptide Tyr-Gln-Gln-Pro-Val-Leu-Gly-Pro-Val-Arg-Gly-Pro-Phe-Pro-Ile. Peptides with a molecular weight higher than 3 000 Da are not bitter because they cannot interact with the taste receptors.

Methods for eliminating bitter peptides in partial protein hydrolysates are known, but they cause a significant loss of essential amino acids. These procedures usually include additional enzymatic hydrolysis under controlled conditions (a shorter time for the hydrolysis leads to higher peptides that are not bitter) and a selection of suitable proteases, such as aminopeptidases, carboxypeptidases and some other proteases. Enzymes of plant and microbial origin have been successfully used for this purpose. For example, the intracellular peptidases from *Lactococcus lactis* ssp. *cremoris* and *Brevibacterium linens*, which have high proteolytic activity, successfully hydrolyse bitter peptides in cheeses.

The bitterness of hydrolysates can be reduced considerably by the plastein reaction. Under certain conditions (temperature, pH, peptidase concentration and other factors) proteases (such as endopeptidases) can synthesise new larger peptides from undesirable bitter peptides; these larger peptides are known as plasteins and are not bitter. The plastein reaction involves a transpeptidation and/or condensation mechanism. The plastein synthesis can also

[5]Proteolysis has three phases (see Section 2.4.5.2.1): proteolysis in milk before cheese manufacture due to indigenous milk protease (plasmin) activity, the enzymatically induced coagulation of the milk in rennet cheeses (hydrolysis of κ-casein by rennin) and proteolysis during ripening of most cheeses, which is the most important reaction having a major impact on flavour and texture.

be used to incorporate nutritionally valuable amino acids (methionine, lysine and tryptophan) into proteins of low biological value, for the removal of undesirable amino acids from proteins (phenylalanine, see Section 10.3.1.1.3) and for the recovery of protein from non-traditional and waste proteinaceous materials.

Cyclic peptides

Cyclic dipeptides, 2,5-dioxopiperazines, are mainly formed by dehydration of linear dipeptides as a result of the hydrolysis of proteins and also partly through stepwise cyclisation from free amino acids. They have been identified in several fermented and thermally treated foods and sometimes contribute to the perceived bitterness. 2,5-Dioxopiperazines are partly responsible for the bitterness of coffee, cacao, roasted cereal grains, roasted malt, beer, aged sake and other foods. For example, in roasted coffee the following proline-based diketopiperazines have been identified: cyclo(Pro-Ile), cyclo(Pro-Leu), cyclo(Pro-Phe), cyclo(Pro-Pro) and cyclo(Pro-Val). Acid hydrolysis (traditionally with hydrochloric acid) of protein-rich material (mostly oilseed meal) is employed for the production of hydrolysed vegetable proteins that are used as seasonings. The resulting hydrolysates contain relatively large amounts (about 25 mg/kg) of 2,5-dioxopiperazines derived from the hydrophobic amino acids valine, leucine, isoleucine and phenylalanine. The main products are cyclo-(Val-Val), cyclo-(Val-Ile), cyclo-Val-Phe, cyclo-(Leu-Phe) and cyclo-(Ile-Ile), which are formed by dehydration of linear dipeptides under acidic conditions. The 2,5-dioxopiperazines (*Z*)-cyclo(Leu-Pro) and (*Z*)-cyclo(Phe-Pro) have also been found in wheat dough and bread. The levels of these cyclic dipeptides increased from approximately 1 to 35 and 25 μg/kg of dough, respectively, after 48 h of incubation. The bread crumbs and crust contained, respectively, almost 100 and 2 000 times the levels found in the dough prior to baking.

Bitter, off-tasting, cyclopeptides can also arise during the storage of cold pressed vegetable oils. For example, fresh linseed oil provides a nutty flavour, but on storage at room temperature it develops a bitter off-taste. The key bitter compound responsible for the bitter taste is the cyclic octapeptide cyclo-(Pro-Leu-Phe-Ile-Met*O*-Leu-Val-Phe), known as cyclolinopeptide E, which contains methionine sulfoxide.

2.3.3.2.2 Bioactive peptides

Some peptides with particular amino acid sequences, which are inactive in the intact protein, may exert biological functions after their release from the intact protein molecule. Such bioactive peptides have been described as peptides with hormone- or drug-like activity that eventually modulate human physiological functions through binding to specific receptors on target cells, leading to induction of physiological responses. Bioactive peptides (protein fragments), generally consisting of 3–20, but sometimes more, amino acids, arise through hydrolysis of many dietary proteins during:

- fermentation processes using proteolytic starter cultures
- during the manufacture of protein hydrolysates
- during digestion of proteins
- by the action of digestive enzymes on proteins *in vitro*.

Depending on their biological properties, bioactive peptides can be divided, for example, into:

- **opioids**
- **antihypertensive peptides**
- **mineral-binding peptides**
- **antimicrobial peptides**
- **immunomodulating and cytomodulating peptides**.

Unlike endorphins (peptide hormones found mainly in the brain that bind to opiate receptors and reduce the sensation of pain and affect emotions), exorphins are the brain's opiates that are found in food, mainly in milk, dairy products and gluten-rich wheat. The most important exorphins are β-casomorphins (BCMs), which are released by the digestion of β-casein in bovine milk and dairy products. They are denoted by a numeral indicating the number of amino acids. The first β-casomorphin, heptapeptide (also known as casomorphin 7, Tyr-Pro-Phe-Tyr-Gly-Tyr-Ile), was isolated from a casein hydrolysate in 1970. Homologous sequences have also been identified in human milk. In 2009, the European Food Safety Agency (EFSA) published a comprehensive review on casomorphins, which are classified as substances with opioid effects. This is why milk, for instance, can help people fall asleep because it contains opioid peptides. Exorphins also arise from other milk proteins (α-casein – α_{S1}-casein exorphin, κ-casein – casoxins A, B and C, α-lactalbumin – α-lactorphin, β-lactoglobulin – β-lactorphin and serum albumin – serorphin), cereal proteins (such as wheat gluten and gliadin), soybeans (α-protein), meat and poultry proteins (albumin, haemoglobin, γ-globulin), eggs (ovalbumin) and other proteins.

Angiotensin-converting enzyme (ACE) inhibitory peptides represent an additional group of bioactive peptides, which inhibit the activity of the angiotensin-converting enzyme, resulting in a reduction of blood pressure. ACE inhibitory peptides with a chain length of 2–10 amino acids were first obtained from milk proteins (caseins and serum proteins), although they have also been found in a number of other animal and plant proteins. For example, one of about 150 bioactive peptides from α_{S1}-casein (of the genetic variant B, see Section 2.4.5.2.1) is hexapeptide, which is composed of Lys-Thr-Thr-Met-Pro-Leu. An antihypertensive hexapeptide isolated from wheat sourdough, traditionally used in bread making, is composed of amino acids Val-Pro-Phe-Gly-Val-Gly, which occur at the 287–292 position of a low molecular weight glutenin subunit.

Similarly to mineral-binding proteins, casein-derived phosphopeptides can form salts with minerals such as calcium due to the binding properties of the phosphoserine residue. These peptides are involved in the increased absorption and bioavailability of calcium and other minerals (zinc, copper, manganese and iron)

in the intestine. An example is a heptapeptide derived from κ-casein (Pro-Val-Ala-Leu-Ile-Asn-Asn).

Peptides with antioxidant properties are effective against enzymatic and non-enzymatic oxidation of lipids, as free radical scavengers and in metal ion chelations. An example is a soybean pentapeptide with the sequence Leu-Leu-Pro-His-His.

Antimicrobial peptides are effective against different bacteria and yeasts. An example of an antimicrobial peptide is lactoferricin, which includes residues 17–41 from lactoferrin. Other peptides with antimicrobial properties are isracidin, derived from α_{S1}-casein (residues 1–23), and casocidin-I, as a result of hydrolysis of α_{S2}-casein (residues 150–188). Lactoferricin also has immuno- and cytomodulatory properties that similarly show a number of peptides arising by hydrolysis of milk caseins and serum proteins.

The resistance of peptides to gastrointestinal digestion is an important prerequisite in order to obtain physiological effects *in vivo* after oral administration of bioactive peptides. Current knowledge indicates that absorption is only possible with dipeptides and tripeptides, which are probably absorbed through passive diffusion through the intestinal mucosa, although the amounts absorbed are extremely small. The absorbed peptides are further degraded by peptidases in the blood. For example, the major constituent in human blood after oral ingestion of gelatin hydrolysates was dipeptide Pro-Hyp, in addition to small amounts of Ala-Hyp, Ala-Hyp-Gly, Pro-Hyp-Gly, Leu-Hyp, Ile-Hyp and Phe-Hyp, which suggests that oral administration of gelatin (collagen) may be involved in some biological activities in the body. In the case of opioids, passage through the blood–brain–barrier is necessary in order for them to act on the central nervous system.

2.3.4 Properties

2.3.4.1 Physical-chemical properties

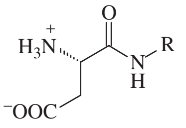
As with amino acids, peptides dissociate in aqueous solutions and form internal salts (zwitterions). The values of the dissociation constants (pK values) and isoelectric points (pI) of some simple peptides are shown in Table 2.6. It is clear (by comparing the values for Gly, Gly-Gly and Gly-Gly-Gly) that the increase in the molecular weight of a peptide reduces the acidity and basicity of the functional groups. The pI values of peptides are lower than those of the corresponding amino acids. The pK and pI values of peptides containing the same amino acids are different depending on the order of the amino acids (in Gly-Asp and Asp-Gly).

2.3.4.2 Organoleptic properties

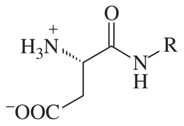
Peptides, like amino acids, can taste bitter, sweet, salty or indifferent. Most natural and synthetic oligopeptides have a bitter taste (see Section 2.3.3.2). A sweet taste indicates dipeptides derived from L-aspartic acid (**2-91**) and others derived from its lower homologue L-aminomalonic acid (**2-92**). R^1 is always a hydrogen atom or a methyl group, substituents R^2 are alkyls or aryls and substituents R^3 are esterified carboxyl groups (usually methyl esters, but some ethyl, propyl, isopropyl and other esters are also sweet). The best known of these compounds is the methyl ester of L-aspartyl-L-phenylalanine, which has long been used as the sweetener aspartame (see **11-27**).

Table 2.6 pK and pI values of peptides.

Peptide	pK_1	pK_2	pK_3	pK_4	pK_5	pI
Gly-Gly	3.12	8.17	-	-	-	5.6
Gly-Gly-Gly	3.26	7.91	-	-	-	5.6
Ala-Ala	3.30	8.14	-	-	-	5.7
Gly-Asp	2.81	4.45	8.60	-	-	3.6
Asp-Gly	2.10	4.53	9.07	-	-	3.3
Lys-Ala	3.22	7.62	10.70	-	-	9.2
Asp-Asp	2.70	3.40	4.70	8.26	-	3.0
Lys-Lys	3.01	7.53	10.05	11.01	-	10.5
Lys-Lys-Lys	3.08	7.34	9.80	10.54	11.32	10.9

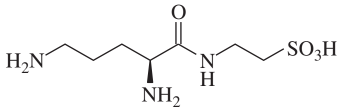


2-91, aspartyl dipeptides

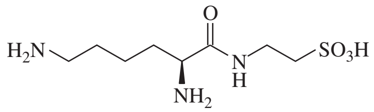


2-92, aminomalonyl dipeptides

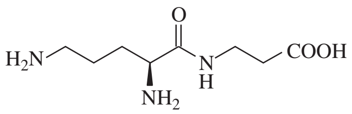
The hydrochlorides of some dipeptides have a salty taste, which suggests the possibility of using them as a replacement for sodium chloride for people who need to cut down their salt intake for health reasons (see Section 8.3.2). L-Ornithyl taurine (**2-93**) and L-lysyl taurine hydrochlorides (**2-94**) also exhibit a salty taste of similar quality and intensity as sodium chloride. A weaker salty taste is found in the hydrochloride of L-ornithyl-β-alanine (**2-95**) and the hydrochloride of L-ornithyl-γ-aminobutyric acid.



2-93, ornithyl taurine



2-94, lysyl taurine



2-95, ornithyl-β-alanine

2.4 Proteins

The process of protein synthesis creates polymers from amino acids. Proteins commonly contain more than 100 amino acids bound into linear chains by covalent peptide bonds. Their relative molecular weight ranges from 10 000 to several million daltons. In addition to peptide bonds that link the amino acids in a protein chain, some other covalent bonds (such as disulfide bonds, between bound cysteine side chains, and ester bonds, allowing connection of serine, threonine, arginine or lysine through phosphoric acid) can be important determinants of protein structure. Apart from covalent bonds, various electrostatic interactions can also be important to the protein structure (ionic, hydrogen and hydrophobic interactions; see Section 7.6.1). Ionic bonds are formed between amino acids bearing opposite electrical charges and are found mainly in the hydrophobic core of the protein. Hydrogen bonds (bridges) can exist between a variety of atoms, for example between two different amino acid side chains, or between an amino acid side chain and a water molecule. Hydrophobic bonds occurring in the hydrophobic protein core are the major forces driving the correct folding of the protein. Protein molecules also bond with water and various inorganic ions. Some proteins contain physically or chemically bound organic compounds, such as lipids, sugars, nucleic acids and various coloured organic compounds.

Apart from water, proteins form the major portion of the mass of living organisms. According to the biological functions they perform in biochemical processes, proteins are often distinguished as:

- **structural** proteins (occurring primarily as structural components of cells and animal and plant tissues)
- **catalytic** proteins (enzymes, hormones)
- **transport** proteins involved in the movement of ions, small molecules or macromolecules (e.g. haemoglobin)
- **mobility** proteins participating in muscle contraction (actin, myosin and actomyosin)
- **defence** proteins (antibodies and immunoglobulins)
- **storage** proteins (e.g. ferritin)
- **sensory** proteins (rhodopsin)
- **regulation** proteins (histones and hormones).

Proteins ultimately perform **nutritional** functions, together with lipids and carbohydrates, and are among the most important components of human dietary requirements. They are our main source of nitrogen, a source of essential amino acids and of material needed for the growth and repair of muscles, bones, skin and other tissues, and are a source of energy. For human nutrition, proteins are derived from various foods. These mainly come from foods of animal origin (meat, milk and eggs), a category that on average represents about 25% of protein in the human diet worldwide. Proteins of vegetable origin (mainly cereals and pulses, but also fruits, vegetables and root crops) contribute about 65% of the per capita supply of protein on a worldwide basis.[6]

Non-conventional sources such as seeds, plant waste and algae (mainly algae of the genera *Chlorella*, *Spirulina* and *Scenedesmus*) can also potentially provide protein for human consumption.

In food raw materials, which are, with some exceptions, post-mortem animal tissues and post-harvest plant tissues, the majority of proteins do not possess the envisioned biological functions. Furthermore, animal and plant tissues of raw materials are often damaged during food processing or cooking, which sometimes results in desirable, but also undesirable, activity of various enzymes. Apart from the enzymes that occur naturally in raw materials, foods may also contain enzymes produced by microorganisms (both naturally occurring and also those whose properties are used in food technology procedures), and also enzymes added for various reasons during processing. In many cases foods are heat processed, during which there are a number of physical and chemical changes to the proteins, which are known by the general term **denaturation**. Thus proteins are often classified according to the state in which they are found in foods as **native** (natural) proteins and **denatured** proteins. Native proteins preserve all the biological functions they have in living organisms, while denatured proteins do not, as the quaternary, tertiary and secondary structures present in their native state are lost. Both native and denatured proteins are the main nutritional sources of protein. The intentional modification of food protein side chains with chemical reagents, currently applied to only a limited extent, produces chemically **modified** proteins with altered properties. The modified proteins, mostly used as food additives for specific purposes, can have improved nutritional quality, physical states (such as texture) and functional properties (e.g. whipping capacity). Whilst many such modifications offer opportunities for improving food proteins and extending their availability from non-conventional sources, careful considerations of their safety and acceptability is required.

2.4.1 Classification and nomenclature

As with all macromolecules, protein nomenclature predominantly uses trivial names. Proteins can be classified from a variety of perspectives, which reflect their importance in nutrition, origin, structure, chemical and biochemical properties and other attributes.

The amount of protein that the body is able to absorb and use from a certain protein source varies, because protein from one food may be absorbed better than a protein from a different source, even if the same amounts of each are provided. According to their bioavailability (see Section 2.4.4.2), the following protein categories can be identified:

- **fully available**, which contain all essential amino acids at levels required for human nutrition (e.g. egg and milk proteins)

[6]Animal (and plant) protein supplies per capita (%) for selected geographic regions in 1989 were: Western Europe 60 (42), North America 73 (34), Latin America 29 (57), Africa 12 (79), Middle East 17 (78), and Far East 11 (81).

- **partly available**, in which some essential amino acids are present in somewhat lower amounts than required (e.g. meat proteins)

- **non-available**, in which essential amino acids are present in much lower amounts than required (e.g. all vegetable proteins and animal proteins from connective tissues).

Depending on their structure (or the presence of non-protein components), native proteins are divided into:

- **simple** proteins

- **conjugated** proteins.

Simple proteins that consist of amino acids only are also traditionally separated into two basic groups according to the shape of the molecule (to a certain extent this classification coincides with a further classification into soluble and insoluble proteins):

- **globular** proteins or spheroproteins (e.g. albumins and globulins), where the molecules have a round to spherical shape, non-polar functional groups are within the molecule, the polar functional groups form the outer core and bind to water molecules, generally soluble in water or dilute salt solutions, forming colloidal solutions (see Section 7.6.3.5) and many act as enzymes;

- **fibrillar** proteins or scleroproteins (for example, practically insoluble structural proteins collagen, keratin and elastin) the molecule of which has the shape of macroscopic fibres.

Depending on the proportion of non-protein compounds covalently bound to the proteins, conjugated proteins are divided into several groups:

- **nucleoproteins**, which contain ester-bound nucleic acids;

- **lipoproteins** are proteins conjugated with neutral lipids, phospholipids, steroids (such as cholesterol) and mostly occur in egg yolk and blood plasma;

- **glycoproteins** containing bound saccharides, mostly oligosaccharides (e.g. the structural protein collagen in meat, κ-casein in milk and some egg white proteins), saccharides are bound by an *O*-glycosidic bond to serine, threonine or 5-hydroxylysine;

- **phosphoproteins** containing bound phosphoric acid (α- and β-caseins in milk and the egg yolk protein phosvitin);

- **chromoproteins** containing bound porphyrin or flavin derivatives (haemoglobin, myoglobin, ferritin, peroxidases, catalases and dehydrogenases with NAD and FAD cofactors);

- **metalloproteins** that contain metals bound by coordinate bonds (examples include the protein ferritin, which is the storage form of iron in the liver, or ceruloplasmin, which contains bound copper, these proteins represent the most important sources of iron and copper in human nutrition).

In the past proteins were classified according to their solubility, but use of this classification is now limited. However, many trivial names of proteins are still based on this classification:

- **soluble** proteins

- **insoluble** proteins, which include fibrillar proteins (scleroproteins).

The soluble proteins are divided into the following six groups:

- **albumins** are neutral proteins soluble in water, salted out from aqueous solutions by ammonium sulfate at a saturation of 60%, at 75 °C, irreversibly coagulating, and include lactalbumin in milk, ovalbumin and conalbumin in egg white, leucosin in wheat, legumelin in lentils and peas and phaseolin in kidney beans;

- **globulins** are weakly acidic proteins insoluble in water, but soluble in dilute salt solutions, e.g. in 5% sodium chloride, in acids and bases, salted out by ammonium sulfate at a saturation of 40% and coagulating on heating; globulins soluble in salt-free water are **pseudoglobulins**, those insoluble in salt-free water are **euglobulins**; examples include muscle proteins myosin and actin (also the product of their interaction, actomyosin), lactoglobulin in milk, ovoglobulin in egg white, edestin in wheat, avenalin in oats, legumin and vicilin of lentils, peas and other legumes, conglycinin and glycinin in soybeans, tuberin in potatoes, almond protein amandin, Brazil nuts protein excelsin and hemp protein edestin;

- **prolamins** are proteins insoluble in water, but soluble in dilute solutions of salts, acids and bases and in 70% ethanol, do not coagulate on heating, and primarily include vegetable lysine-free proteins containing higher amounts of bound proline and glutamine, an examples are gliadin in wheat and rye, secalin in rye, hordein in barley, gliadin in oats, oryzin in rice and zein in maize;

- **glutelins** are insoluble in water but soluble in dilute solutions of salts, acids and bases, differ from prolamins in that they are insoluble in ethanol and coagulate on heating, they contain a considerable amount of glutamic acid; the most common glutelin is glutenin found in wheat, which is responsible for some of the baking properties of bread wheat; examples of other glutelins are rye secalinin, barley hordenin, rice oryzenin and maize zeanin;

- **protamines** are basic proteins soluble in water, dilute acids and in ammonium hydroxide and do not coagulate on heating; they contain large amounts of basic amino acids (about 80% arginine), occur in fish sperm (e.g. cyprimine in carp, salmine in salmon, sturine in sturgeon, clupeine in herring and scombrine in mackerel;

- **histones** are basic proteins soluble in water and dilute acids, but not in ammonium hydroxide and do not coagulate on heating, they contain higher amounts of lysine, arginine and histidine and are present at high levels in the nuclei of animal and plant cells, where they are bound to nucleic acids.

2.4.2 Structure

In practice, to characterise proteins in terms of food and nutrition, information on the overall amino acid composition is usually sufficient. In many cases, however, detailed knowledge of the protein structure is also required. Four levels of the protein structure are recognised: **primary**, **secondary**, **tertiary** and **quaternary**. Detailed descriptions of the structure of proteins can be found in biochemistry textbooks.

2.4.2.1 Primary structure

The primary structure of proteins includes, for example, the data on the structure of covalently bound amino acid molecules, the number and order (sequence) of amino acids in the peptide chain, the character of basic peptide bonds, number, character and position of other covalent bonds (disulfide bridges and other bonds).

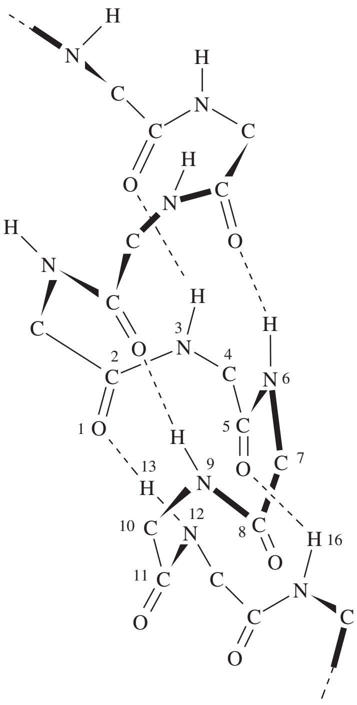


Figure 2.18 α-Helix (C_α atoms are shown without substituents).

2.4.2.2 Secondary structure

The secondary structure of a segment of a polypeptide chain is the local spatial arrangement of its main chain atoms, without considering the conformation of its side chains or its relationship to other segments. The polypeptide chains of native proteins have particular secondary structures in different parts of the chain. A certain spatial arrangement (conformation) of the chain is given by its primary structure (amino acid sequence), fixed by non-covalent interactions of functional groups of the amino acids. Hydrophobic interactions are present when the amino acids have hydrophobic side chains, amino acids with an electric charge in the side chain (acidic and basic hydrophilic amino acids) are involved in electrostatic interactions and other hydrophilic and amphiphilic amino acids can form hydrogen bonds through their functional groups. There are three common secondary structures in proteins, namely **helices**, **sheets** and **turns**.

The most important elements of the secondary structure (conformation of the polypeptide chain) are helical structures arising from the coiling of peptide chains (or part of a chain) in the neighbourhood of the C_α into a spiral known as a helix. Helices have characteristic torsion angles, numbers of amino acid residues per turn and helix heights. These characteristics are described by values of n_m, where n is the number of amino acid residues per turn and m is the number of atoms in the ring, including the hydrogen atom forming a hydrogen bond. Helices are chiral and can be right-handed or left-handed. Only right-handed helices are found in natural proteins (with some exceptions, for instance in collagen).

The major secondary structure of a protein is the right-handed α-**helix** (Figure 2.18). An α-helix is a rigid arrangement of a polypeptide chain, in which intramolecular hydrogen bonds play a significant role. If the helix has a translation of 0.15 nm along the helical axis and a pitch height (the vertical distance between one consecutive turn of the helix) of 0.54 nm, then the helix has 3.6 amino acid residues per turn (0.54/0.15 = 3.6). The average size of an α-helix is 11 amino acid units, which corresponds to about three turns of the helix. The C=O group of the peptide bond of an amino acid with a sequence number n is bound by hydrogen bonding to the N–H group of the peptide bond of an amino acid with a sequence number n+4. This α-helix is also known as a 3.6_{13} helix as the hydrogen bond is between atoms 1 and 13 of the polypeptide chain. This secondary structure can be found in at least part of any polypeptide chain in the majority of proteins (in myoglobin, collagen and other proteins).

A rarer type is a helix 2.2_7 (the hydrogen bond is between atoms 1 and 7 of the polypeptide chain). A right-handed helix 3_{10} (three units per turn, pitch height 0.6 nm), which is narrower and also steeper than an α-helix (the hydrogen bond is between atoms 1 and 10 of the polypeptide chain), sometimes occurs as an α-helix junction with another part of the polypeptide chain. The so-called helix 4.4_{16} (π-helix) only exists at the end of several helices (4.4 units per turn, pitch height 0.52 nm, the hydrogen bond is between atoms 1 and 16 of the polypeptide chain).

β-**Structures** are somewhat less common regular secondary structures of proteins, also known as β-pleated sheets or just β-sheets (Figure 2.19). These are characterised by long extended polypeptide chains in contrast to the compact helices. These structures are formed by combining parallel or antiparallel oriented polypeptide chains (β-strands) that line up and form bridges of extramolecular hydrogen bonds (unlike the helices, which

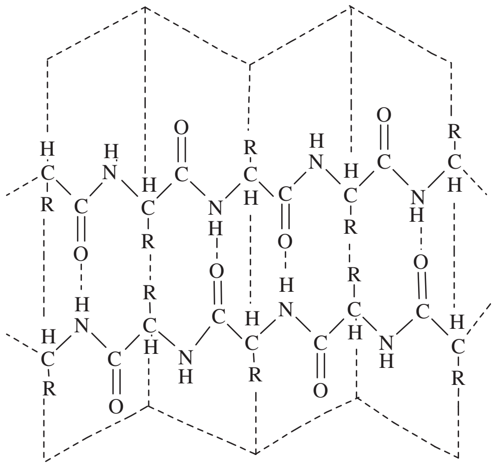


Figure 2.19 Antiparallel β-sheet consisting of two β-strands.

are bound by intramolecular hydrogen bonds), creating a stable β-sheet. On average, a β-sheet consists of six β-strands, and structures with less than five β-strands are rare. Each polypeptide chain contains up to 15 amino acid residues. Some globular proteins rarely produce a parallel structure; an antiparallel structure occurs more frequently, and the polypeptide chains containing proline, such as the collagen polypeptide chain, never form β-structures. A large closed β-sheet (typically having an antiparallel structure) is called a β-barrel. β-Barrels are found in proteins known as porins that occur in the cytoplasmic membrane of gram-negative bacteria and in the outer membranes of mitochondria and plastids.

There are many structural motifs that are formed by the combination of helices and sheets, but the common types of protein secondary structures (helices and pleated sheets) represent about half of the common globular proteins structures. These combinations require turns in the protein structure that reverse the direction of the peptide chain. These so-called β-turns are considered to be a third common secondary structure motif. Approximately one third of all the residues in globular proteins are found in turns. Turns are almost always found on the surface of proteins and often contain Pro and/or Gly. The remaining segments of the molecule have the so-called non-repetitive structure (random coils, turns and bulges), which is also highly organised and characterised in terms of the backbone conformation and hydrogen bonding.

Fibrillar proteins have a specific secondary structure. For example, the basic secondary structure of keratin consists of two pairs of closely linked right-handed α-helices (a superhelix) that are coiled into a left-handed helix. The basic secondary structure of collagen is a triple helix in which the left-handed polypeptide helix is coiled into the right-handed superhelix.

2.4.2.3 Tertiary and quaternary structures

The tertiary structure of a protein molecule is the arrangement of all its atoms in space (conformation) without regard to its relationship with neighbouring molecules or subunits. It describes the packing of the individual sections of the peptide chains (α-helices, β-sheets and random coils) in the whole polypeptide chain with respect to each other, as these secondary structure units are not planar, but may be bent, coiled and connected together in different ways. Various types of covalent bonds (such as disulfide bridges) and non-covalent interactions between functional groups of amino acids (hydrophobic and electrostatic interactions, see Section 7.6.1) participate in the binding of individual protein sections and in the full fixation of the tertiary structure.

The tertiary structures of many proteins are now known. One example is the myoglobin molecule, which is composed of 153 amino acids, of which about 80% are involved in α-helical structures (Figure 2.20). Its tertiary structure is that of a typical water soluble globular protein. The polypeptide chain is coiled into eight separate right-handed α-helices (designated A through H) that are connected by short non-helical regions. The size of each helix ranges from 7 (helix D) to 26 amino acid residues. In total, 121 amino acids out of 153 occur in helical structures. The last coils of helices A, C, E and G are reinforced by helices 3_{10}. The hydrophobic cleft (hydrophobic pocket), containing one haem prosthetic group with an iron atom, is formed largely by helices E and F and is then in contact with helices B, C, G and H and turns CD and FG. The fifth ligand of the coordination bond of Fe^{2+} is histidine F8, the eighth amino acid in the α-helix F, which is known as proximal histidine. The so-called distal histidine (histidine E7) bonds oxygen to oxymyoglobine via a hydrogen bond (see Section 9.2.1.1). In deoxymyoglobine the sixth binding positions of Fe is vacant.

Many protein molecules do not consist of just one but of several identical or different polypeptide chains that form a complex structure, which is known as the quaternary structure of a protein. The quaternary structure is the arrangement of protein subunits in space and the association of its inter-subunit contacts and interactions,

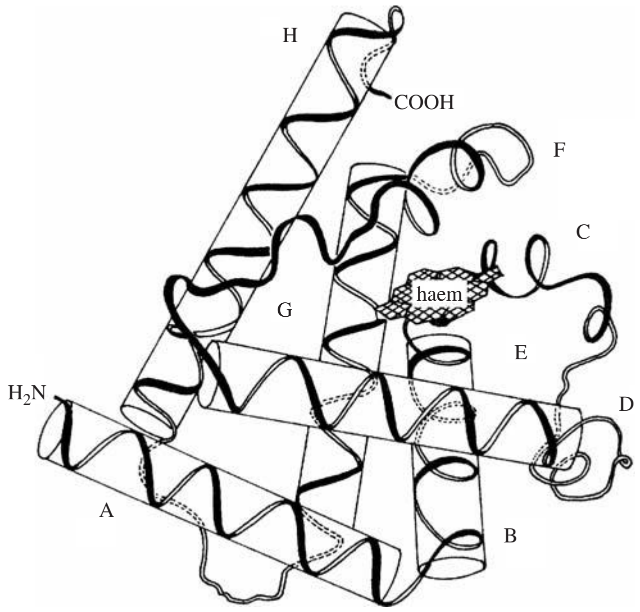


Figure 2.20 Structure of muscle tissue protein myoglobin.

without regard to the interior geometry of the subunits. The subunits in a quaternary structure must be in non-covalent association. Therefore, a quaternary structure only exists if there is more than one polypeptide chain present in a complex protein. For example, avidin in eggs (68 300 kDa) and haemoglobin in blood (64 500 kDa) consist of four subunits, while β-lactoglobulin in milk (36 000 kDa) is composed of two subunits (monomers).

Each subunit of a haemoglobin tetramer has a haem prosthetic group with Fe^{2+}, which is identical to that described for myoglobin. The common peptide subunits are designated α, β, γ and δ. Both the α and β subunits have structural characteristics similar to that of myoglobin.

The subunit structure of β-lactoglobulin of cows' milk is shown in Figure 2.21. This protein (18 kDa) occurs in milk and in a solution of pH 5–7.5 it is generally a dimer, in a solution of pH 3.5–5 it is an octamer and in a solution of pH 3.5 it is a monomer. The monomer polypeptide chain consists of 162 amino acid residues. It contains two disulfide bridges (Cys 66-Cys 160 and Cys 106-Cys 119), one free thiol group (Cys 121), the *N*-terminal amino acid is leucine and the *C*-terminal amino acid is isoleucine. In the milk of other mammals, there are genetic variants of several amino acids, which differ in structure. The polypeptide skeleton is composed of nine β-strands (they are marked with arrows pointing to the *C*-terminus of the molecule), which form a cylindrical β-barrel. The strands contain the following amino acid residues: A 16–27, B 39–44, C 47–58, D 62–76, E 80–84, F 89–97, G 102–109, H 115–124 and I 145–150. The connection between the strands H and I is made through an α-helix, which contains amino acid residues 130–140. Strand I participates in dimer formation, which is based on an antiparallel hydrophobic interaction with another monomer, between isoleucine 29 of one monomer and isoleucine 147 of a second monomer.

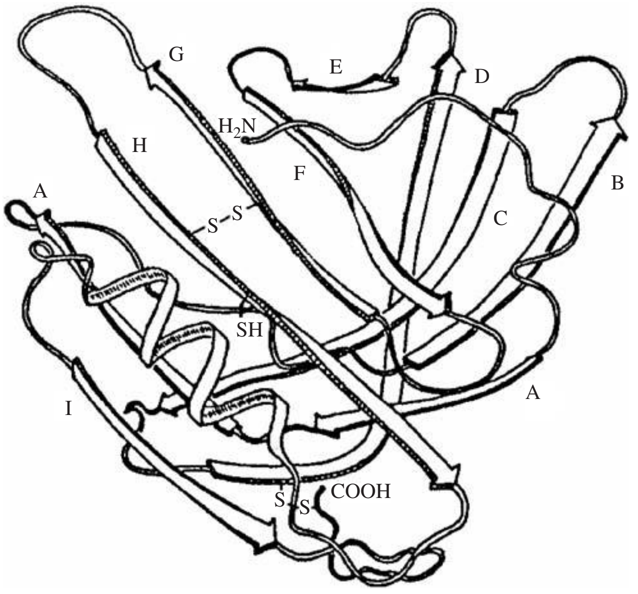


Figure 2.21 Structure of β-lactoglobulin of cow's milk.

2.4.3 Properties

2.4.3.1 Dissociation and hydration

Globular proteins are soluble in polar solvents such as water and in aqueous solutions of acids and bases. Structural fibrous proteins are insoluble in water. Prolamins also dissolve in less polar solvents such as ethanol. In addition to the protein structure, solubility depends on the relative permittivity of the solvent, the pH value of the solution (the minimum solubility is at, or in the vicinity of, the isoelectric point), its ionic strength (a low concentration of salt increases the solubility by the salting-in effect, higher concentrations of salt reduces the solubility by the salting-out effect; see Section 7.6.3.2.3), temperature and other factors.

Proteins dissociate in solutions, as do amino acids or peptides, to form macromolecular polyions. Therefore, proteins are polyampholytes. Depending on the pH of a solution, protein molecules are positively or negatively charged ions as a result of the dissociation of the functional groups of the various amino acids, especially basic amino acids (such as lysine) and acidic functional groups (aspartic and glutamic acids) in the side chains. The net charge (the difference between the number of positive and negative charges) is therefore positive or negative depending on the pH value. The pH at which the protein has no net charge is the isoelectric point (the negative logarithm is the p*I* value). It depends on the type of protein and lies in the pH range 2–11. It should be noted that the protein still contains charged side chains at its isoelectric point; however, at the isoelectric point, the number of positively charged side chains is equal to the number of negatively charged side chains.

Hydration dictates a range of processes, including protein folding, ligand binding, macromolecular assembly and enzyme kinetics. For example, solutions of globular proteins are colloidal dispersions (see Section 7.6.3.2). The protein molecule has the character of a molecular micelle with hydrophobic amino acids arranged towards the interior of the molecule, whereas hydrophilic amino acids are bound outwards. The polar core is hydrated in aqueous solutions, which explains the solubility of globulins. The amount of bound water (monomolecular layer; see Section 7.6.4.2) is about 0.2–0.5 g per 1 g of protein. Dispersions of proteins are therefore hydrophilic colloids. Other layers of water are now bound less tightly. Proteins in aqueous solutions are usually molecular dispersions as the dispersed phases are neutral molecules or ions. Most protein solutions are monodisperse colloidal systems, they contain single molecules, but some proteins are oligodisperse colloidal systems that contain dimers and higher oligomers. For example, β-lactoglobulin molecules form in a solution of pH 3.5–5 octamers and in a solution of pH 5–7.5 dimers. Some proteins form aggregates of molecules, known as micellar colloids (e.g. α-, β- and κ-casein in milk). The mechanical, kinetic, optical and electrical properties of disperse systems of proteins (sols and gels) are described in Section 7.6.3.2.4.

2.4.3.2 Denaturation

Native proteins are precisely folded polypeptidic chains. The denaturation of proteins involves the disruption and possible destruction

of the secondary, tertiary and quaternary structures under the mild effects of various physical factors and in the presence of chemical agents. Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure (sequence of amino acids) remains the same after the denaturation process. However, the long-term and severe effects of some factors that led to the denaturation can also cause changes to the primary structure (degradation of the polypeptide chain to shorter units and possibly to free amino acids during the cooking of meat). The conformational changes may be reversible or irreversible. Native and denatured proteins are in equilibrium and the original conformations can sometimes be restored, returning the protein to its original condition. This process is call renaturation (Figure 2.22). Changes in protein conformation, however, are usually irreversible. Loss of biological activity and the original function of the protein is thus a result of these changes.

Denaturation disrupts the normal α-helices and β-sheets in a protein and uncoils it into less organised and random shape structures. New (previously unavailable) functional groups can interact with water and therefore many proteins in the denatured state have an increased ability to bind water (30–45%). Denaturation is often accompanied by protein coagulation, which is due to aggregation of protein molecules, because functional groups of the proteins react with themselves, thus reducing the number of functional groups available to enter into interactions with water. Hence, the ability of proteins to bind water decreases (see Section 7.6.3.5). For example, the proteins in eggs denature and coagulate during cooking. Denatured proteins can also react with other food components, for example with saccharides in the Maillard reaction.

Various physical factors can cause denaturation (mostly changes in temperature, pressure, exposure to ultrasound and penetrating electromagnetic radiation). Denaturation as a result of chemical agents occurs, for example, in the presence of salts, acids and bases or in the presence of surfactants. For example, heat can be effectively used to disrupt hydrogen bonds and non-polar hydrophobic interactions. This occurs because heat increases the kinetic energy and causes the molecules to vibrate so rapidly and violently that the bonds are disrupted. The activation energy of denaturation by heat is dependent on temperature and on other factors such as the amount of water present, and is very high compared with enzymatic reactions. For example, the activation energy of thermal denaturation of the milk proteins β-lactoglobulin and α-lactalbumin during pasteurisation (at 80 °C) is approximately 270 kJ/mol. During sterilisation (at >100 °C), the activation energy of denaturation is about 60 kJ/mol. The denaturation of fibrillar proteins in meat requires a significantly higher activation energy and proceeds in a different way. An example is the thermal denaturation of collagen in the production of gelatine, which requires long, intense heating procedure in the presence of water.

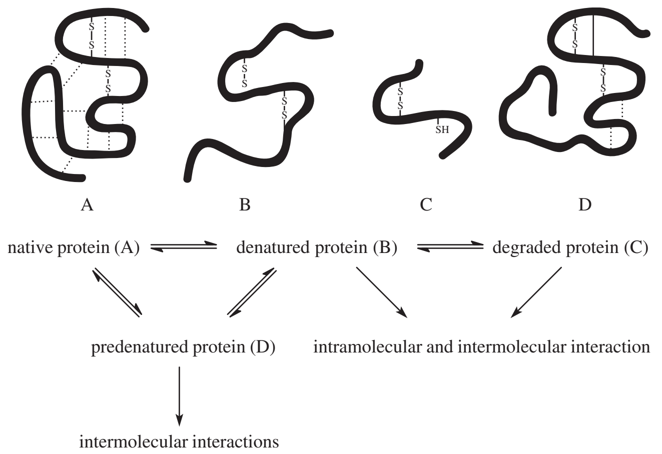


Figure 2.22 Termal denaturation of proteins.

The denaturation of proteins does not just occur during thermal processing of foods, but also can be the result of low temperatures (when food is exposed to freezing temperatures of between 0 and −15 °C). As a result, crystals of ice are produced, which can break the cell membranes, followed by denaturation due to surface phenomena at the interface of the two phases (protein solution and ice crystals), or possibly due to the freezing of water needed to maintain the native conformation of the protein. Loss of water also leads to an increase in the salt concentration and osmotic pressure, which can accelerate protein denaturation. Lipoproteins are particularly sensitive to denaturation (e.g. those in egg yolk). The extent of the denaturation is limited in rapid freezing at very low temperatures, however, as smaller ice crystals are formed.

Denaturation of globular proteins also occurs at the interface of a protein with another phase (solid, liquid or gaseous). As a result of conformation changes, the molecule unfolds, the hydrophilic groups are oriented into the aqueous phase and hydrophobic groups into the less polar phase. This leads to partial denaturation of proteins in the presence of surfactants, for example in emulsions and foams (see Section 7.6.3.2.3). When whipping egg whites, partial denaturation occurs due to the mechanical stress, through the action of shear forces.

From the nutritional point of view, denaturation is usually desirable since denatured proteins are more amenable to digestive enzymes than the native proteins. Denaturation, therefore, increases the bioavailability of proteins (e.g. sulfur-containing amino acids in cereals and legumes). Some antinutritional and natural toxic substances are also denatured, such as protease inhibitors (see Section 10.2.1.1), lectins (see Section 10.2.2.6), enzymes and other unwanted proteins and undesirable microorganisms.

2.4.4 Physiology and nutrition

2.4.4.1 Fate of proteins in human organisms

The human organism is not able to use dietary proteins as such. They must be hydrolysed into single amino acid molecules before they can be absorbed. The hydrolysis of proteins (mostly denatured proteins) is catalysed by proteolytic enzymes called proteases (proteinases or peptidases), which have relatively high substrate specificity. They catalyse the hydrolysis of interior peptide bonds to form peptides of different sizes (endopeptidases such as pepsin, trypsin and chymotrypsin) or attack the terminal amino acids (exopeptidases). Hydrolysis of the *N*-terminal amino acids is

catalysed by aminopeptidases while carboxypeptidases hydrolyse amino acids from the *C*-terminus.

Protein digestion begins in the stomach. The gastric juices contain the endopeptidase pepsin created by the peptic cells of the gastric mucosa. Pepsin is released from the proenzyme pepsinogen in the acid medium of the stomach (pH 2), which is provided by the hydrochloric acid also secreted by the gastric mucosa. The major component of pepsin in adult mammals is pepsin A.[7] Protein hydrolysis by pepsin just gives smaller units, polypeptides. Digestion of polypeptides continues in the upper portion of the small intestine, the duodenum, through the action of the proteolytic enzymes trypsin and chymotrypsin, which are produced in the pancreas as the inactive proenzymes known as trypsinogen and chymotrypsinogen, respectively. They act in an alkaline medium in the small intestine and break down polypeptides into smaller peptides, which are hydrolysed to their component amino acids by a mixture of aminopeptidases and dipeptidases (known as erepsin) released by the mucosa. Free amino acids are absorbed across the intestinal mucosa into the lymphatic circulatory system and from there into the bloodstream.

The bloodstream provides a readily available pool of amino acids, which can be taken up by the liver and cells of various organs to support the reactions that are essential for life. The liver is the major site for synthesis of tissue proteins and for the conversion of excess amino acids into many other products. After deamination, the carbon skeleton enters the citric acid cycle and is used for the production of glucose (gluconeogenesis) and fatty acids and to generate energy through the oxidation of acetate into carbon dioxide and water. The resulting ammonia is converted into urea and excreted by the kidneys. This process overloads the body; therefore excessive protein intake, which could be used as a source of energy, is disadvantageous.

D-Amino acids that occur in dietary proteins and peptides are eliminated from the body in urine (10–20%) and the remaining D-amino acids are catabolised by the flavoenzyme D-amino acid oxidase to 2-oxo acids in the liver, kidneys and brain.

Tissue proteins are hydrolysed by thiol proteases called cathepsins (denoted by the letters B, D, L, H, S, M and T). They also act in post-mortem autolysis of proteins during meat curing.

Proteases have found practical application in various food and other technologies; for instance, rennin is used in the dairy industry for the manufacture of hard cheeses, while plant proteases, such as papain, are employed in the prevention of protein hazes in beer.

2.4.4.2 Nutrition

Dietary proteins are essential nutrients that provide adequate amounts of amino acids to allow the body to synthesise its own proteins and other nitrogen-containing compounds and serve as a source of energy. On average, proteins contain 16% nitrogen by weight.[8] Dietary protein requirements are determined by the need for essential amino acids and nitrogen, intake of other nutrients (carbohydrates and fat for energy intake), body weight, physical activity and so on. Specific needs include growth, pregnancy, lactation, illness or injury. The Recommended Dietary Allowances (RDA values) are: 1.5 g/kg/day for infants, 1.1 g/kg/day for 1–3 year old children, 0.95 g/kg/day for 4–13 year old children, 0.85 g/kg/day for 14–18 year old adolescents, 0.8 g/kg/day for adults and 1.1 g/kg/day for pregnant and lactating women. On average, based on the RDA data, the average male who weighs 70 kg should consume approximately 56 g of protein per day, while the average female who weighs 50 kg should consume approximately 40 g of protein per day. Many nutritional experts feel the RDA for protein is far too low and is only suitable for sedentary adults. For teenagers, active adults and some other people, the amount of dietary protein should be higher (1.2–1.8 g/kg). Research does not support protein intake in excess of 2.0 g/kg body weight. Excess protein intake is associated with body dehydration and may be related to excessive urinary calcium losses and inadequate carbohydrate intake. Inadequate protein intake can lead to disturbances in mental and physical development, decreased resistance to infection, deterioration of wound healing after injury and other problems.

The **energy yield** from proteins is 17 kJ/g (4 kcal/g), roughly the same as for carbohydrates (with the exception of sugar alcohols). The energy yield from fat (triacylglycerols) is about 37 kJ/g (9 kcal/g). Sugar alcohols, some organic acids and ethanol can also be used as a source of energy by the human body.

Nutrition recommendations indicate how much protein is needed by considering daily energy intake and the percentage of energy that should come from protein. Although carbohydrates are the body's preferred fuel source, approximately 20–30% of energy requirements should be derived from proteins. The U.S National Academy of Sciences has recommended that between 45 and 65% of energy should be derived from carbohydrates, 20–35% from fat and 10–35% from protein, with no more than 25% of the total energy being from added sugars. Recommendations by the American Heart Association and the American Diabetes Association fall well within these broad guidelines: approximately 50% of energy from carbohydrates, 30% from fat and 20% from protein. In other words, if the average male who weighs 70 kg consumes 9350 kJ (2200 kcal) per day and 20% of the energy is derived from protein, this amount of energy corresponds to 110 g of protein daily (1.6 g/kg body weight). The ratio of animal and vegetable protein should be about 1:1.

2.4.4.3 Protein nutritional value

The nutritional value of a food is mainly determined by the amount of protein it contains and the quality of that protein. Dietary protein quality depends on how well the essential amino acid content

[7] Rennin (chymosin) is another proteolytic enzyme secreted by the stomach of newborn mammals. Its role in digestion is to curdle (coagulate) milk in the stomach, which is a process of considerable importance in a very young animal. Aside from its physiological role, chymosin is also a very important industrial enzyme because it is widely used in cheese making.

[8] The amino acid contents of foods is often related to 16 g of nitrogen (i.e. 100 g of pure protein containing 16% of nitrogen). The nitrogen content of pure proteins and foods is in fact slightly different, meat and eggs contain 16% of nitrogen, egg white has a nitrogen content of 15%, gelatine 18%, milk and dairy products 15.7%, cereals (wheat, rye, barley and oats) 17.2%.

matches the requirements of the human body, on the availability of peptide bonds to digestive enzymes (protein digestibility) and how efficiently the amino acids are absorbed into the body for utilisation. Proteins from animal sources provide all of the essential amino acids in adequate amounts and are called **complete** (**whole**) **proteins**. Proteins of plant origin tend to be deficient in one or more of the essential amino acids and are termed **incomplete proteins**. The human body uses dietary essential amino acids in specific ratios to each other, so if a person does not get enough of one amino acid compared with the others, these other acids can only be used at a level comparable to that of the lower acid.

Proteins have different biological availabilities in the human body and a number of methods have been introduced to evaluate and measure protein utilisation and retention. Two types of measurements are used to estimate protein quality: biological assays and chemical analysis. The most common measures of protein quality included Biological Value (BV), Protein Efficiency Ratio (PER), Net Protein Utilisation (NPU), Amino Acid Score (AAS; also known as chemical score, CS), Essential Amino Acid Index (EAAI) and Protein Digestibility Corrected Amino Acids Score (PDCAAS), amongst other procedures and modifications.

The biological value is the proportion of nitrogen retained in the body compared with that of the nitrogen absorbed (taking no account of digestibility). A dietary protein that is completely utilisable (a complete or whole protein), that is, ingested, absorbed and incorporated into body proteins (such as eggs and human milk) has a BV = 90–100, while gelatine has BV = 0 as it does not contain the essential amino acid tryptophan (Table 2.7). The protein efficiency ratio is determined by dividing the weight gain of a test subject who had been given certain proteins by the intake of that particular protein during the test period. The net protein utilisation is the ratio of nitrogen retained (converted into proteins) to nitrogen supplied.

Table 2.7 Comparison of various methods for the evaluation of nutritional value.

Food	BV	NPU	PER	PDCAAS
Beef meat	74-80	67-73	2.7-2.9	0.92-0.94
Fish	76-83	78-80	2.7	0.9-1.0
Milk (cow)	84-91	81-82	2.5	1.00
Eggs (whole)	93-100	88-94	3.1-3.9	1.00
Whey protein	96	92	3.0	1.00
Casein	77-80	76	2.5	1.00
Wheat	54-64	67	0.8	0.37-0.54
Rice	83	40	1.5	0.54-0.78
Soybeans	73-74	48-61	2.3	0.91
Potatoes	73	60	1.8	0.71-0.82
Gelatine	0	0	0	0

Table 2.8 Essential amino acid contents in a reference protein (in mg/g protein) and their recommended daily intakes (RDI) in mg/kg body weight.

Amino acid	Protein FAO/WHO	RDI	Amino acid	Protein FAO/WHO	RDI
Valine	32	26	Threonine	27	15
Leucine	55	39	Lysine	51	30
Isoleucine	25	20	Phenylalanine/ tyrosine	47	25
Methionine/ cysteine	25	15	Tryptofan	7	4

Table 2.9 Amino acid content of meat, chicken and fish (in g per 16 g of nitrogen).

Amino acid	Beef	Pork	Pork offal	Mutton	Horse	Chicken	Fish
Ala	5.8	5.5	6.1	6.6	5.4	3.4	6.0
Arg	6.3	6.4	6.4	6.9	7.2	5.6	5.7
Asx	9.0	8.9	8.2	8.8	8.3	9.2	10.4
Cys	1.3	1.1	1.4	1.3	1.3	1.3	1.2
Glx	15.3	14.5	11.7	14.8	12.2	15.0	14.1
Gly	4.9	5.7	-	5.9	4.3	5.3	4.8
His	3.4	3.3	2.6	2.7	2.8	2.6	3.5
Ile	4.8	5.1	6.1	5.0	6.5	5.3	4.8
Leu	8.1	7.6	8.3	7.7	9.5	7.4	7.7
Lys	8.9	8.1	8.5	8.2	10.0	8.0	9.1
Met	2.7	2.7	2.5	2.5	2.8	2.5	2.9
Phe	4.4	4.2	4.8	4.0	3.8	4.0	3.9
Pro	3.8	4.6	5.3	4.7	4.0	4.1	3.7
Ser	4.0	4.2	4.7	4.2	4.2	3.9	4.3
Thr	4.6	4.9	4.5	4.7	3.9	4.0	4.6
Trp	1.1	1.4	1.3	1.3	1.0	1.0	0.6
Tyr	3.6	3.6	3.4	3.3	3.7	3.3	3.7
Val	5.0	5.2	6.0	5.1	5.0	5.1	6.1
Total EAA[a]	44.5	43.8	46.8	42.9	47.2	41.9	45.0
Total AA[b]	97.0	96.8	98.5	97.4	95.7	91.0	97.5
EAAI (%)[c]	80	81	78	81	69	79	80
AAS (%)[d]	69	69	71	67	63	64	70
Limiting AA	Val	Ser	Ser	Ser	Trp	Trp	Trp

[a] EAA = essential amino acids.
[b] AA = amino acids.
[c] EAAI = essential amino acid index.
[d] AAS = amino acid score (for limiting amino acids).

Table 2.10 Amino acid content of milk proteins (in g per 16 g of nitrogen).

Amino acid	Milk[a] Cow	Goat	Sheep	Human	Amino acid	Milk[a] Cow	Goat	Sheep	Human
Ala	3.5	2.7	3.2	3.9	Pro	9.1	8.3	10.8	7.1
Arg	3.3	1.3	2.9	3.9	Ser	5.8	4.1	5.6	4.7
Asx	7.7	7.6	7.0	8.5	Thr	4.5	4.4	3.7	4.5
Cys	0.8	1.6	1.4	1.3	Trp	1.4	1.3	1.9	1.8
Glx	22.2	18.3	22.1	15.2	Tyr	4.8	3.2	5.0	3.3
Gly	2.0	1.4	1.9	2.5	Val	5.8	6.5	6.2	4.5
His	2.7	3.6	2.5	2.5	Total EAA[b]	47.2	41.7	45.8	40.1
Ile	4.7	5.2	4.6	4.1	Total AA[c]	103.5	89.0	101.8	88.5
Leu	9.5	9.2	9.3	8.8	EAAI (%)[d]	100	99	60	69
Lys	7.8	5.2	7.2	6.8	AAS (%)[e]	75	73	53	53
Met	2.5	1.3	1.6	1.6	Limiting AA	sulfur and Ile	sulfur and Lys	sulfur and Ile	sulfur and Ile
Phe	5.4	3.8	4.9	3.5					

[a]Colostrum (milk produced by the mammary glands in late pregnancy) has a different composition.
[b]EAA = essential amino acids.
[c]AA = amino acids.
[d]EAAI = essential amino acid index.
[e]AAS = amino acid score (for limiting amino acids).

The amino acid (chemical) score is expressed in milligrams of an amino acid in 1 g of the test protein divided by milligrams of the same amino acid in a reference protein. The essential (limiting) amino acid that has the lowest AAS value thus determines the nutritional value of a protein. The reference protein is a protein of high nutritional value (such as β-lactalbumin) with balanced amounts of all essential amino acids. The AAS value for each of them is 100%. The composition of the reference protein is shown in Table 2.8, which also gives the recommended daily intake of essential amino acids in adult humans. These values depend on nutritional and health status, age and other individual factors, and have undergone considerable revision in recent years. The AAS value only takes one amino acid into account. More accurate data on the nutritional value of proteins are provided by the index of essential amino acids, which includes the contributions of all the essential amino acids as the geometric mean of the AAS values. The calculated AAS and EAAI values for meat, chicken and fish are given in Table 2.9, for milk in Table 2.10, for eggs in Table 2.11, for cereals and pseudocereals in Table 2.12, and for legumes, oilseeds and nuts in Table 2.13. The protein digestibility corrected amino acids score is a more recent method to evaluate protein quality. It is based on both the amino acid requirements of humans and their ability to digest it. It is expressed in milligrams of limiting amino acid in 1 g of test protein divided by milligrams of the same amino acid in 1 g of the reference protein (which corresponds to AAS), and multiplied by faecal digestibility percentage (Table 2.7).

In human nutrition, which assumes a varied and well balanced diet, the evaluation of the nutritional quality of proteins is not

Table 2.11 Amino acid content of hen's egg proteins (in g per 16 g of nitrogen).

Amino acid	Egg Whole	Egg white	Yolk	Amino acid	Egg Whole	Egg white	Yolk
Ala	5.9	6.1	5.5	Phe	5.7	6.0	4.2
Arg	6.1	5.7	7.5	Pro	4.2	3.6	4.3
Asx	9.6	11.0	10.6	Ser	7.6	7.3	9.0
Cys	2.4	2.4	1.7	Thr	5.1	4.8	5.5
Glx	12.7	12.2	14.0	Trp	1.6	1.4	1.9
Gly	3.3	3.6	3.1	Tyr	4.2	3.5	4.0
His	2.4	2.4	2.5	Val	6.8	4.8	7.2
Ile	6.3	6.1	5.1	Total EAA[a]	51.3	47.9	48.4
Leu	8.8	8.3	8.5	Total AA[b]	107.1	99.8	104.9
Lys	7.0	6.6	7.7	EAAI (%)[c]	100	94	95
Met	3.4	4.0	2.6	AAS (%)[d]	100	71	74

[a]EAA = essential amino acids.
[b]AA = amino acids.
[c]EAAI = essential amino acid index.
[d]AAS = amino acid score (for limiting amino acids), none limiting amino acid.

Table 2.12 Amino acid content of cereal and pseudocereal proteins (in g per 16 g of nitrogen).

Aminokyselina	Wheat	Rye	Barley	Oats	Rice	Maize	Millet	Buckwheat	Amaranth
Ala	3.6	4.3	4.0	4.5	6.0	7.5	7.9	4.7	3.4
Arg	4.6	4.6	4.7	6.3	8.3	4.2	5.3	9.8	7.4
Asx	4.9	7.2	5.7	7.7	10.3	6.3	8.0	8.9	8.3
Cys	2.5	1.9	2.3	2.7	1.1	1.6	2.4	2.4	1.4
Glx	29.9	24.2	23.6	20.9	20.6	18.9	18.6	17.3	15.4
Gly	3.9	4.3	3.9	4.7	5.0	3.7	3.8	5.0	8.7
His	2.3	2.2	2.1	2.1	2.5	2.7	2.4	2.1	2.3
Ile	3.3	3.5	3.6	3.8	3.8	3.7	4.1	3.4	3.6
Leu	6.7	6.2	6.7	7.3	8.2	12.5	9.6	5.9	5.3
Lys	2.9	3.4	3.5	3.7	3.8	2.7	3.4	3.8	5.0
Met	1.5	1.5	1.7	1.7	2.3	1.9	2.5	1.5	1.8
Phe	4.5	4.4	5.1	5.0	5.2	4.9	4.8	3.8	3.6
Pro	9.9	9.4	10.9	5.2	4.7	8.9	6.1	4.3	3.6
Ser	4.6	4.3	4.0	4.7	5.4	5.0	4.9	5.0	7.1
Thr	2.9	3.3	3.3	3.3	3.9	3.6	3.9	3.6	3.5
Trp	0.9	1.0	0.9	1.1	0.8	0.7	2.0	1.4	1.5
Tyr	3.0	1.9	3.1	3.3	3.5	3.8	3.2	2.4	3.4
Val	4.4	4.8	5.0	5.1	5.5	4.8	5.5	6.7	4.3
Total EA[a]	32.8	31.6	35.8	37.1	38.5	40.2	41.1	34.8	28.4
Total AA[b]	96.5	92.0	94.6	93.3	101	97.5	98.1	93.3	89.4
EAAI (%)[c]	68	75	78	79	76	55	67	76	76
AAS (%)[d]	44	46	54	57	57	41	53	51	54
Limiting AA	Lys	Trp, Ile	Lys, Leu	Ile, Lys	Ile, Lys	Lys	Lys	Lys, Ile	Lys Ile

[a] EAA = essential amino acids.
[b] AA = amino acids.
[c] EAAI = essential amino acid index.
[d] AAS = amino acid score (for limiting amino acids).

particularly important. An exception may be some extreme diets (such as those of vegans), which might not supply some essential amino acids in sufficient quantities. The evaluation of protein quality in feeds for livestock and poultry is important in cases requiring rapid muscle growth or high yields of milk or eggs. In these cases, feed is often enriched (fortified) with limiting amino acids, mostly lysine and methionine, which increase its nutritional value.

2.4.5 Occurrence, composition and changes

Foods differ considerably in their protein contents, and the protein contents vary in their amino acid compositions and quality. The protein contents can effectively vary anywhere between 0 and 100% of the dry matter The richest sources of protein are mainly foods of animal origin (Table 2.14). For example, the amount of protein in dried egg whites approaches 100% whereas refined vegetable oil does not contain any protein at all. However, some products from plant origins, especially legumes (peas, beans, lentils) and oilseeds (soybeans, peanuts, poppy seeds, nuts) are also good protein sources. Cereals and cereal products have medium protein content, while vegetables, fruits and root crops have a low protein content (Table 2.15). Vegetable oils, vinegar and sugar contain only traces or no protein at all.

2.4.5.1 Meat, meat products, poultry and fish

Animal cells produce four main types of tissue:

- **epithelial** tissue
- **connective** (supporting) tissue

- **muscle** tissue
- **nervous** tissue.

Epithelial tissues cover the whole surface of the body and the digestive tract. Connective tissues are present in cartilages and bones; adipose tissue is also a connective tissue. Muscle tissue covers the bones and is the building material of the gut. Nervous tissue is a major part of the nervous system, which is divided to the central nervous system (nervous tissue of the brain and spinal cord) and the peripheral nervous system. Blood is often classified as an additional tissue. Almost all tissues of animals are used as food for humans, especially the meat of mammals, poultry and fish. The term meat generally means the skeletal muscle and associated fat and tissues, but it may also describe other edible tissues such as organs and offal of mammals, poultry and fish. A typical consumer connects the very vague term meat with a product that, besides muscle tissue, contains a certain proportion of epithelial and connective tissue (such as skin, fat, cartilage and bones). The main types of muscles that are used as food are skeletal muscles, the smooth muscles of the digestive tract and internal organs and cardiac muscle tissue.

In addition to water and proteins, meat commonly contains about 1.5% fat (although in some meats this can be much more, such as pork belly which can contain >50% fat), about 1% minerals and small or trace amounts of carbohydrates. The glycogen content in muscle tissue is 1–2%, but its amount decreases very rapidly post-mortem and meat usually contains only a small part of the original amount, a typical concentration is about 0.2%. Meat also contains sugar phosphates (mainly glucose 6-phosphate,

Table 2.13 Amino acid content of legumes, oilseeds and nuts (in g per 16 g of nitrogen).

Amino acid	Soybeans	Lentils	Peas	Beans	Sunflowerseed	Peanuts	Sesame seed	Walnuts	Hazel nuts
Ala	4.3	4.3	4.1	4.2	4.2	3.9	4.5	4.1	4.2
Arg	7.2	8.7	9.5	5.7	8.0	11.2	12.1	12.3	15.0
Asx	11.7	11.6	11.0	12.0	9.3	11.4	8.2	8.3	7.2
Cys	1.3	0.9	1.1	0.8	1.5	1.2	1.8	0.5	0.4
Glx	18.7	16.6	16.1	14.8	21.8	18.3	19.4	20.1	20.5
Gly	4.2	4.2	4.0	3.8	5.4	5.6	4.9	7.0	8.7
His	2.5	2.7	2.3	2.8	2.3	2.4	2.4	2.0	1.8
Ile	4.5	4.3	4.3	4.2	4.3	3.4	3.6	3.9	6.2
Leu	7.8	7.6	6.8	7.6	6.4	6.4	6.7	7.5	6.2
Lys	6.4	7.2	7.5	7.2	3.6	3.5	2.7	1.6	2.9
Met	1.3	0.8	0.9	1.1	1.9	1.2	2.8	1.3	0.8
Phe	4.9	5.2	4.6	5.2	4.4	5.0	4.4	4.1	3.6
Pro	5.5	4.3	3.9	3.6	4.5	4.4	3.7	4.7	5.6
Ser	5.1	5.3	4.3	5.6	4.3	4.8	4.7	6.1	9.6
Thr	3.9	4.0	4.1	4.0	3.7	2.6	3.6	2.7	2.7
Trp	1.3	1.5	1.4	1.4	1.4	1.0	1.0	1.0	1.1
Tyr	3.1	3.3	2.7	2.5	1.9	3.9	3.1	3.1	3.7
Val	4.8	5.0	4.7	4.6	5.2	4.2	4.6	4.4	6.4
Total EAA[a]	39.3	39.8	38.2	38.6	34.1	32.4	34.8	26.5	33.1
Total AA[b]	98.5	97.4	93.4	90.9	93.9	94.2	94.7	94.5	106.7
EAAI (%)[c]	62	41	50	47	93	69	63	60	35
AAS (%)[d]	47	31	37	34	56	43	43	24	22
Limiting AA	sulfur, Val	sulfur, Trp	sulfur, Trp	sulfur, Trp	Lys, sulfur	sulfur, Ile	Lys, Ile	sulfur, Lys	sulfur, Lys

[a] EAA = essential amino acids.
[b] AA = amino acids.
[c] EAAI = essential amino acid index.
[d] AAS = amino acid score (for limiting amino acids).

Table 2.14 Protein content in foods of animal origin.

Food	Content (%) (from-to)	Content (%) (mean)	Food	Content (%) (from-to)	Content (%) (mean)
Meat, meat products			*Game*	20.8–24.3	22.8
Beef meat	13.1–27.0	20.8	Deer	22.7–23.2	23.0
Veal meat	18.3–28,0	21.8	***Fish***	16.0–29.0	18.7
Pork meat	9.1–20.2	15.5	Carp	17.7–17.9	17.8
Sheep meat	14.9–18.0	16.4	Trout	20.2–20.8	20.5
Rabbit meat	19.8–20.3	20.1	Cod	17.8–17.9	17.8
Offal	10.4–22.7	17.2	***Milk, dairy products***		
Pork liver	21.1–21.7	21.4	Cow milk	3.0–3.4	3.2
Beef liver	20.2–20.5	20.4	Curd	18.0–20.6	19.4
Sausages	12.8–28.0	20.8	Soft cheeses	12.5–20.2	15.0
Poultry			Hard cheeses	23.8–40.6	24.8
Chicken	21.2–21.4	21.3	Butter	0.4–0.6	0.5
Turkey	19.2–19.8	19.5	***Eggs***	12.5–12.6	12.6
Duck	11.2–11.8	11.5	Egg white	10.8–11.0	10.9
Goose	15.1–16.7	15.9	Egg yolk	15.8–16.0	16.0

Table 2.15 Protein content in foods of plant origin.

Food	Content (%) (from-to)	Content (%) (mean)	Food	Content (%) (from-to)	Content (%) (mean)
Cereals, cereal products			*Vegetables*		
Wheat flour	8.1–12.8	10.1	Fruit vegetables	0.7–1.7	1.2
Rye flour	5.1–12.0	9.6	Brassica vegetables	0.7–1.8	1.4
Rice (peeled)	7.0–7.3	7.1	Leaf vegetables	1.3–3.9	2.6
Bread (wheat)	6.7–11.4	6.7	Root vegetables	1.0–3.3	2.0
Bakery products	7.3–9.7	8.5	*Fruits*		
Pastry	3.5–7.8	5.6	Raw	0.3–1.5	1.0
Pasta	9.8–12.5	11.8	Dried	1.4–4.0	2.3
Legumes, oilseeds, nuts	21.4–44.7	24.2	***Other foods***		
Soybeans		44.7	Mushrooms		2.6
Lentil		25.8	Yeast		10.6
Poppy seed		18.0	Cocoa		18.0
Potatoes		2.0	Chocolate	4.9–8.1	6.8

Table 2.16 Muscle proteins.

Protein	Proportion (%)	Protein	Proportion (%)
Myofibrillar proteins	60.5	***Sarcoplasmatic proteins***	29.0
Myosin	29	Enzymes	24.5
Actin	13	Myoglobin	1.1
Connectin	3.7	Haemoglobin and other extracellular proteins	3.3
Tropomyosin	3.2	***Structural proteins and proteins of organelles***	10.5
Troponin (C, I, T)	3.2	Collagen	5.2
Actinin (α-, β-, γ-)	2.6	Elastin	0.3
Myomesin, desmin	5.8	Mitochondrial proteins	5.0

see Section 4.2.2.1) in small amounts (0.05–0.2%) and some free sugars (mainly glucose).

Muscle proteins in typical mammalian muscle tissue constitute around 20% of the muscle weight. The major proportion of muscle is made up of muscle fibre proteins (elongated, threadlike cells) called **myofibrillar proteins**. Smaller amounts of soluble **sarcoplasmatic proteins** and insoluble **structural proteins** from connective tissue are also present (Table 2.16). Myofibrillar and sarcoplasmatic proteins are almost complete (whole) proteins, while the nutritional value of structural proteins is very low as they are almost indigestible. Table 2.17 gives the amino acid composition of some pure animal proteins; Table 2.9 presents the amino acid compositions for the main types of meat proteins.

In addition to proteins, the other nitrogen compounds present in meat are free amino acids. The level of the individual amino acids is about 0.005% (their total amount is 0.1–0.3%); alanine (about 0.01%), glutamic acid (about 0.05%) and taurine (0.02–0.1%) occur in somewhat higher concentrations, followed by histidine dipeptides and guanidine compounds such as creatine and creatinine. Important groups of nitrogenous compounds are purine and

Table 2.17 Amino acid content in pure animal proteins (in g per 16 g of nitrogen).

Amino acid	Actin[a]	Myosin[a]	Collagen[a]	Elastin[a]	Keratin[b]	α-Casein[c]	α-Lactalbumin[c]	β-Lactoglobulin[c]	Ovalbumin[d]
Ala	6.1	9.3	11.0	21.1	5.0	4.9	2.6	9.2	8.9
Arg	6.3	5.4	4.9	1.2	7.2	2.8	0.7	1.9	3.9
Asx	10.4	8.6	5.0	1.0	6.0	7.6	15.0	9.9	8.2
Cys	1.3	1.5	0.0	0.3	11.2	0.5	5.7	1.9	1.8
Glx	14.2	19.3	7.6	2.4	12.1	17.9	9.3	15.4	13.2
Gly	4.8	3.2	31.4	25.5	8.2	3.4	4.6	2.2	4.8
His	2.8	2.0	0.5	0.1	0.7	2.4	2.0	1.2	1.8
Hyl	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
Hyp	0.0	0.0	10.1	1.5	0.0	0.0	0.0	0.0	0.0
Ile	7.2	5.3	1.2	3.7	2.8	6.1	5.6	6.2	6.3
Leu	7.9	10.0	2.8	8.6	6.9	7.6	9.4	13.7	8.3
Lys	7.3	10.4	2.6	0.5	2.3	8.0	8.4	9.3	5.1
Met	4.3	2.9	0.5	traces	0.5	2.2	0.7	2.5	4.1
Phe	4.6	3.4	1.6	5.9	2.5	3.5	2.9	2.5	5.5
Pro	4.9	2.1	11.8	11.6	7.5	9.0	1.8	5.2	3.7
Ser	5.6	5.3	3.8	0.9	10.2	7.6	4.8	4.1	9.1
Thr	6.7	5.5	2.0	1.1	6.5	4.4	4.9	4.9	4.0
Trp	2.0	0.5	0.0	0.0	1.2	1.2	3.7	1.5	0.7
Tyr	5.6	2.4	0.3	1.3	4.2	5.0	3.2	2.5	2.4
Val	4.7	2.8	2.1	16.5	5.0	6.0	4.3	6.0	7.1

[a] Beef meat protein.
[b] Sheep wool protein.
[c] Cow milk (whey) protein.
[d] Hen egg (white) protein.

pyrimidine nucleotides, nucleosides and free bases (0.1–0.25%). Rigor mortis usually sets in between 2 and 4 hours after death and within approximately 1 hour post-mortem the main nucleotide in meat is ATP. After rigor mortis dissipates, 5′-inosinic acid (also known as 5′-inosine monophosphate or 5′-IMP) predominates at a level of 0.02–0.2%, this being formed by the enzymatic decomposition of ATP. The inosinic acid then gradually decomposes during the storage of meat to inosine and hypoxanthine. In addition to IMP, ATP and its breakdown products, ADP and AMP, and other purines and pyrimidines (mainly NAD, 5′-GMP, 5′-CMP and 5′-UMP) occur in small quantities. The other compounds present in significant amounts are numerous organic acids. The main acid is lactic acid, and in fresh meat the content ranges from 0.2 to 0.8%. Glycolic acid (about 0.1%) and succinic acid (about 0.05%) are also present at relatively higher levels, and other acids of the citric acid cycle in lower amounts. Another important group of nutrients present in meat are vitamins. In general, meat is a good source of all vitamins, particularly of water soluble vitamins.

2.4.5.1.1 Myofibrillar proteins

The basic structural unit of skeletal muscle is a muscle fibre composed of long, cylindrical cells of 10–100 μm in diameter and lengths of up to several tens of mm (usually 20–30 mm). Each muscle fibre is actually a muscle cell containing 100–200 nuclei and normal cell organelles. The muscle fibre is covered by a thin, extensible, semi-permeable membrane, the sarcolemma (or myolemma), which transmits electrical impulses from the nerves. It consists of a cell membrane (plasma membrane) and an outer coat made up of a thin layer of polysaccharide material with numerous thin collagen fibrils. The sarcoplasm of a muscle fibre is comparable to the gel-like cytoplasm of other cells.

Muscle cells contain contractile elements, **myofibrils**, which are essentially bundles of proteins found in the sarcoplasm (Figure 2.23). Muscle myofibrils contain isotropic sections (I-bands about 0.8 μm long) and anisotropic sections (A-bands about 1.5 μm long). The I-band is interrupted by a Z-line about 80 nm wide. The low-density central part of the A-band of is the H-zone. The M-line is situated in the centre of the H-zone. The entire structural unit of myofibrils, which is the span between two Z-lines, is called the sarcomere. In a relaxed state (relaxed muscle), it is about 2.5 μm long. During muscle contraction (shortening) it is shorter, 1.7–1.8 μm.

Myofibrils are composed of two types of protein microfilaments (microfibers). Strong microfilaments have a diameter of 12–16 nm and a length of 1.5 μm. They are composed of the protein myosin, which exhibits ATPase activity. Thin filaments, with a diameter of 8 nm and a length of 1 μm, are predominantly composed of the protein actin (Figure 2.24). Other components of microfilaments are proteins with regulatory functions such as tropomyosin, troponin and other proteins that are important for the stability of the sarcomer structure (Table 2.11).

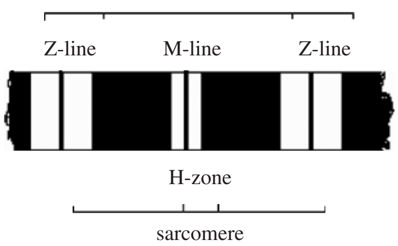


Figure 2.23 Muscle myofibril.

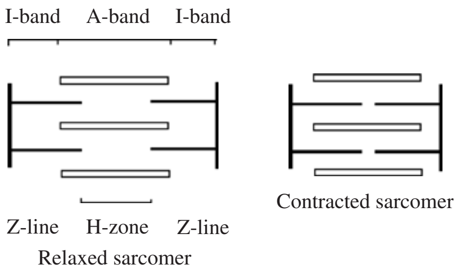


Figure 2.24 Arrangement of actin and myosin (— = actin, ▭ = myosin).

Myosin has a relative molecular weight of about 470 kDa. The molecule contains a fibrillar section, formed by two identical polypeptide chains, largely found in the α-helix conformation. The fibrillar portion of the molecule is connected to a globular head, consisting of four chains with a relative molecular weight of about 20 kDa, which is responsible for ATPase activity of the protein.

Actin is a one-chain globular protein with a relative molecular weight of 43.5 kDa (G-actin, where G stands for globular). This monomeric form polymerises to yield a fibrous form of actin (F-actin, where stands F for fibrous). The units of G-actin are organised into a helix composed of two monomers. This polymer binds to the protein tropomyosin (relative molecular weight 70 kDa), which has a similar structure to the fibrillar part of myosin (two unequal polypeptide chains, essentially curled into an α-helix). The actin is further connected to the regulatory troponins proteins (troponin complexes C, I and T) with relative molecular weights of 18, 24 and 37 kDa, respectively.

In muscle, actin and myosin molecules are able to associate to form a protein complex, actomyosin. Calcium ions lead to this association, and ATP causes the dissociation of the actomyosin. These are the fundamental processes required for muscle activity (contraction and relaxation). The primary process during muscle contraction is the release of calcium ions (e.g. from the sarcoplasmic reticulum) initiated by a nerve impulse. Calcium ions bind to troponin, changing the formation of the molecule, which causes a formational change of tropomyosin and consequently of the actin molecule. Actin then reacts with myosin, forming actomyosin, which results in a shortening of the sarcoma, that is, shortening (contraction) of the whole muscle. During the process of muscle relaxation, the actomyosin complex dissociates through the action of ATP, to give actin and a myosin–ATP complex, which hydrolyses to myosin, ADP and inorganic phosphate. Under normal conditions, ATP is synthesised from pyruvic acid, through the process of glycolysis. In extreme cases (during short, intense exercise or post-mortem), formation of ATP is less efficient, as it is a product of anaerobic glycolysis (from glucose or glycogen) via lactic acid.

2.4.5.1.2 Sarcoplasmic proteins

The sarcoplasm of muscle tissue contains on average 1% myoglobin (9-5) in the dry state (Table 2.16). Its main role is to facilitate the transport of oxygen by diffusion in muscle *in vivo*. In the muscles of aquatic mammals, where its content is about ten times higher than in the muscles of terrestrial mammals, myoglobin also acts as an oxygen reserve. The levels of myoglobin generally depend on the type and origin of the muscle. Besides myoglobin, sarcoplasm contains various enzymes, especially glycolytic enzymes and enzymes from the pentose cycle along with numerous other compounds, such as glycogen, ATP, ATP degradation products and so on.

2.4.5.1.3 Structural proteins

The structural proteins form a specific group of extracellular proteins with protective or supporting roles. They include fibrillar proteins, the most important of which are collagens, elastins and keratins. Their nutritional value is usually low (collagen) or almost nil (elastin, keratin) due to their poor digestibility and inappropriate composition of amino acids.

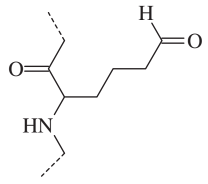
Collagens

Collagens are insoluble extracellular glycoproteins that can be found in all animals. They are the most abundant proteins in the human body (29 different types can be identified). Collagens occur in nearly all connective tissues (skin, cartilage, tendons, filaments and bones). Type I collagen is the main component of tendons, ligaments, skin and bones. Type II collagen is the main component of cartilage.

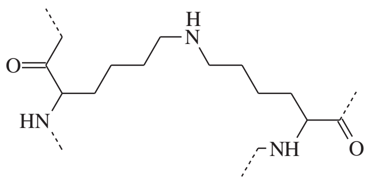
The fundamental structural unit of collagen is the tropocollagen molecule (relative molecular weight about 300 kDa, thickness 1–2 nm and length of ≤300 nm), which consists of a helical structure of three polypeptides, each with molecular weight of about 95 kD. Type I collagen is the most abundant and consists of two chains of α_1 collagen and one chain of α_2 collagen, each having a left-handed helix formation. These three helices are twisted together into a unique right-handed triple helical structure (a heterotrimer known as a super helix), which is stabilised by hydrogen bonds. With type I collagen, each triple helix of tropocollagen naturally aggregates with other triple helices to form a collagen microfibril (thickness <5 nm and length ≤500 nm), which is a right-handed super-super-coil. Cross-linking of collagen microfibrils (catalysed by lysyl oxidase) produces collagen fibres (thickness ≤500 nm and length ≤10 mm). Type I collagen molecules give tissues their mechanical strength and provide the major scaffolding units for the attachment of cells and macromolecules (such as integrins, fibronectin, fibromodulin and decorin). In bones and dentin, the type I collagen is mineralised with hydroxyapatite crystals.

The amino acid composition of collagen is anomalous in comparison with other proteins. Collagen contains high amounts of glycine (30%), proline (about 12%) and also contains hydroxyproline (10%) and 5-hydroxylysine (about 0.5%, Table 2.17). The amino acid sequence is composed of repeating units of Gly-XY, where X is often proline and Y is hydroxyproline or hydroxylysine. The free hydroxyl groups of the peptide chains (derived primarily from hydroxylysine) contain glucose or galactose bound by glycosidic bonds. For example, one fragment of the molecule is 2-*O*-α-D-glucosyl-*O*-β-D-galactosylhydroxylysine. Sugars represent between 0.4 and 12% of collagen molecules by weight. Collagens in connective tissues are accompanied by proteoglycans.

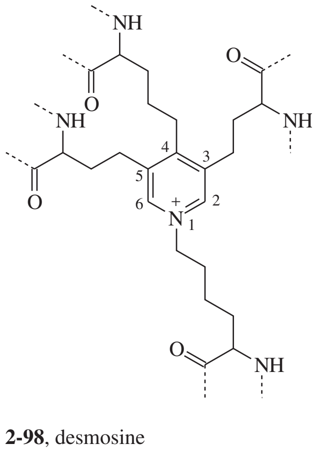
With age, the structure of collagen is stabilised by covalent cross-links. These are formed not only by disulfide bridges (collagen contains low amounts of cysteine, unlike keratin), but also by cross-links from the side chains of lysine, hydroxylysine and histidine. For example, the enzyme lysyl oxidase[9] catalyses the oxidation of lysine (hydroxylysine is oxidised analogously) to the corresponding ω-aldehyde, that is, adipic acid monoaldehyde, or allysine (**2-96**). Two molecules of allysine generate an aldol, which then reacts with the side chains of lysine and histidine to produce imine; these compounds are then stabilised by a reduction or an oxidation. For example, the reaction with lysine followed by imine reduction yields lysinonorleucine (**2-97**). Condensation of three allysine structures in a side chains with the side chain of bound lysine produces cyclic structures derived from pyridine (substituted in positions 1, 3, 4 and 5 of the nucleus). Such structures are desmosine (**2-98**), dihydrodesmosine, tetrahydrodesmosin or pyridines substituted in other positions. The formation of these structures is one of the causes of toughness in meat. Desmosine is also present in elastin.



2-96, allysine



2-97, lysinonorleucine



2-98, desmosine

[9]Lysyl oxidase is inhibited by β-aminopropionitrile formed in some vetchling seeds (*Lathyrus* spp.), which leads to disturbances in the collagen structure with characteristic distortion of limbs and damage to blood vessels. These diseases are known as osteolathyrism and angiolathyrism, respectively (see Section 10.3.2.7.1).

Collagen is hydrolysed by proteases known as collagenases. Collagen denatured in muscle by lactic acid is hydrolysed during the maturation of meat by other enzymes, such as cysteine proteinase (kathepsin B_1). Heat-denatured collagen is hydrolysed by pepsin and trypsin.

Collagen does not dissolve in cold water, salt solutions or dilute solutions of acids and bases. Its characteristic feature is the contraction (shortening of the molecule) when heated to a certain temperature, which is also observed on cooking meat. This form is sometimes referred to as collagen B, while native collagen is known as collagen A. The temperature at which collagen shrinkage occurs depends on its origin. For example, the shrinkage of fish collagen starts at about 45 °C, but mammalian collagen shrinkage occurs at 60–65 °C.

At higher temperatures (approximately 90 °C), the structure of the molecule breaks, the bonds between the polypeptide chains disrupt and each tropocollagen molecule is released to form a soluble sol of gelatine. The sol consists of mixtures of the original polypeptides together with their oligomers and breakdown products. Upon cooling (depending on the concentration of the gelatine and the temperature gradient), a more or less organised structure re-forms again. The new bonds are restored randomly between different parts of the same string or between two strings. The coil–helix transition, followed by aggregation of the helices, leads to the formation of collagen-like right-handed triple-helical proline/hydroxyproline-rich junction zones, similar to the original collagen structure, composed of three polypeptide chains. These structures capture large amounts of water and the gelatine sol forms a gel. Higher concentrations of the proline/hydroxyproline-rich junction zones form stronger gels (see Section 7.6.3.2.3).

Gelatine can also be produced from collagen during cooking, baking and other thermal processing of meat. The extent of gelatinisation depends on the number of cross-links present in the collagen, and therefore also on the age of the animal and the parameters of the thermal process (temperature, time, pressure). Long-term effects of higher temperatures lead to changes in the primary structure of collagen or gelatine (namely, their partial hydrolysis).

Edible gelatine is made from collagen after partial extraction with water, and partial acid (type A gelatine) or alkaline (type B gelatine) hydrolysis. The acid treatment uses pig skin, whereas the alkaline treatment makes use of cattle hides and bones. This gives rise to products with different molecular weights and properties (the alkaline treatment converts asparagine and glutamine residues into their respective acids and results in gelatine with a higher viscosity). Gelatine is primarily used as a gelling agent, forming transparent elastic thermoreversible gels on cooling. In addition, the amphiphilic nature of the molecules endows them with useful emulsification (e.g. in whipped cream) and foam-stabilising properties (as in mallow foam). On dehydration, irreversible conformational changes take place that may be used in the formation of surface films. Gelatine is also used as a clarifiyng agent for wine and fruit juices.

Collagen from the skin dermis is used for the production of collagen casings used in the edible packaging material of sausages. Collagen casings are mainly produced from the corium layer (splits) of cattle hides that consist essentially of collagen (although collagen can also be derived from pigs, poultry and fish). The hides are chopped in water and mixed with lactic acid, which causes them to swell and form a slurry. The acid-swollen slurry is homogenised and filtered to tease the collagen fibres apart. The resultant slurry containing separated collagen fibres is extruded into the casing form. The proteins are then coagulated in a solution of an inorganic salt, plasticised with glycerol, dried, partially re-humidified, wound on reels and shirred.

Elastins

Elastins are insoluble rubber-like proteins conferring elasticity on tissues and organs. Elastin accompanies collagen in a number of connective tissues, undergoing reversible deformations in tendons, blood vessel walls, lung parenchyma, ligaments, skin, tissue membranes and cartilage; it is not hydrolysed by common proteolytic enzymes, but only by the action of specific elastases.

Unlike collagen, elastin is a very flexible net-like structure found in organisms in the form of fibres. The fundamental building component of elastin is the soluble protein tropoelastin that has a relative molecular weight of about 70 kDa and is formed from the soluble precursor, proelastin. Tropoelastin accumulates on the cell surface in small particles that grow into larger spherules (about 1 μm in diameter). The net-like structure of insoluble elastin is based on the association of many cross-linked tropoelastin molecules that are oxidised by lysyl oxidase enzymes at bound lysine molecules, which subsequently undergo aldol condensation and imine formation. One of these cross-links is desmosine (**2-98**) and its isomer isodesmosine (unlike in desmosine, the side chains are in positions 1, 2, 3 and 5 of the pyridine ring). The process of elastic fibre formation includes a number of other molecules. The elastin that is produced is introduced into microfibrils in the extracellular matrix as the central part and interacts with proteins known as fibulins, which form the sheath of these fibres.

Keratins

Keratins are products of epithelial cells. They occur in the outer skin layer (epidermis), and in some skin structures (fur, horns and hooves). The main group are the so-called α-keratins, based on a right-handed polypeptide with an α-helical structure and molecular weight of 10–50 kDa, stabilised by disulfide bridges and hydrogen bonds. Three polypeptides form left-handed helices called protofibrils and 11 protofibrils (two inside and nine outside) create microfibrils. Several hundred microfibrils create macrofibrils that finally form the keratin fibre (e.g. hairs and wool fibres).

Keratins have limited uses in the food industry. In combination with vegetable protein-rich materials, keratin is sometimes used for the production of acid protein hydrolysates (hydrolysed vegetable proteins used as food seasonings). Outside the food industry, alkaline hydrolysis of keratin is used for the production of glues.

2.4.5.1.4 Blood proteins

Blood is a distinct liquid tissue of higher animals, ensuring the transfer of nutrients and metabolic products, gas exchange, metabolic and heat balance. It is also important in the defence reactions of the organism used in self-protection, such as immunological response and coagulation involved in clotting. Blood represents about 5%

of live weight of cattle (cows, calves), 3.3% for pigs and 8% for poultry. In fresh weight, it contains about 80% water (80.5% in beef blood, 79.2% in pork blood) and around 18% protein (17.8% in beef blood and 18.5% in pork blood). About 40% of blood consists of blood cells of which about 94% are erythrocytes containing the blood pigment haemoglobin, 6% thrombocytes and 0.1% leukocytes. About 60% of blood is plasma, which has a dry matter content of 10%. About 6.5–8.5% of this dry matter is albumins and globulins that are present in a ratio of 1.5:1, and 0.3–0.4% of the dry matter is fibrinogen. Blood contains about 0.1% lipids, 1% minerals (mainly phosphates and chlorides of sodium and potassium ions; calcium, magnesium, iron and other ions are present at lower concentrations), small amounts of sugars (0.06–0.07%), free amino acids, vitamins and other low molecular weight nitrogenous substances (urea, creatine and creatinine).

Blood from farm animals is an important raw material for food, feed and industrial use. Clotting of the blood is prevented by binding the calcium ions using citric or phosphoric acids. In the food industry blood drawn aseptically from slaughtered pigs is used to produce some speciality products (e.g. blood sausages and black puddings). Dried blood plasma is used to replace egg whites in baking and in the confectionery industry, while protein fibrin resulting from fibroin can be used for making soup spice preparations (such as acid protein hydrolysates and stock cubes). Dried or cooked blood is also used as feed for livestock and in the production of various veterinary and human medicines and for other technological purposes.

2.4.5.1.5 Changes during storage and processing

After death (post-mortem), a sequence of important biochemical, structural and functional changes occur in the bodies of slaughtered animals when muscle tissue is converted into meat. Although the post-mortem changes proceed in a relatively orderly fashion, a variety of external factors and intrinsic characteristics may accelerate or retard meat maturation and have a marked effect on the quality of the meat. Other important changes occur during thermal processing of meat and during the manufacturing of meat products.

Post-mortem changes and meat maturation

In contrast to tissues *in vivo*, only anaerobic glycolysis takes place in muscles post-mortem, until the muscle glycogen store is depleted and the glycolytic enzymes are inactive. The lactic acid that is formed accumulates in the muscle tissue, which results in a decrease in muscle pH from the initial value of about 6.8 to about 5.8. The glycolytic enzymes are inhibited in this acidic medium and a small proportion of glycogen therefore remains preserved in the muscle. Even after the death of an animal, calcium ions still lead to the interaction of actin with myosin and hydrolysis of ATP to ADP and inorganic phosphate; however, ATP for dissociation of the resulting actomyosin is no longer available. The muscles therefore contain actomyosin, they are shortened (contracted) and the posthumous stiffening (rigor mortis) begins. A muscle, which is soft immediately after the death of the animal, hardens within a few hours. In cattle at normal room temperatures, rigor mortis takes 10–24 hours post-mortem, and this is 4–18 hours in pigs and 2–4 hours in chickens. Rigor mortis then gradually subsides, for example, after 2–3 days post-mortem in beef, or more slowly at lower temperatures. Immediately after killing the animal the meat is dry, and binds its own water and any added water well, as it has a high water binding capacity. In rigor mortis, when lactic acid acidifies the meat, the muscle proteins are present in an environment with a pH around their isoelectric points. The meat is moist-to-wet and its binding capacity is often half or lower than immediately after killing the animal. It is therefore advantageous to produce some meat products before the onset of rigor mortis (in the so-called warm state), otherwise the meat can only be processed after rigor mortis subsides and after maturation, when the pH and binding capacity will increase slightly. To increase the binding capacity of meat, different food additives are used (see Section 11.3.3.1). In practice, meat in rigor mortis cannot be cooked as it remains rigid and does not have the desirable organoleptic properties. During maturation, the cleavage of actomyosin and of other meat proteins is due to the action of specific proteases that include a variety of endogenous enzymes of sarcomers and lysosomal enzymes (cathepsin B, D and L), while colagenases break down collagen fibres. The meat acquires its desirable properties, its texture improves and it becomes suitable for industrial and culinary processing.

Meat defects

Irregularities (defects) of meat known as PSE (pale-soft-exudative meat) and DFD meat (dark-firm-dry meat) occur in pork meat as a result of sensitive animals being subjected to stress. There are a variety of environmental conditions that can cause stress in animals. Some of these include extremes in temperature, humidity, light, sound and confinement. Other stress factors are excitement, fatigue, pain, hunger and thirst. These two defects can occur simultaneously in different types of muscle in the same animal. In beef, although the PSE defect does not occur, a defect similar to DFD meat is seen, known as DCB (dark-cutting-beef), where meat is dark on the cut.

PSE meat is more acidic than normal meat (the pH 1 hour post-mortem is around 5.6 instead of 5.8 and this value lasts much longer than 24 hours). The reason is faster and more extensive glycolysis, resulting in a faster and higher production of lactic acid (glycogen is synthesised from proteins by various mechanisms catalysed by hormones). In the acidic environment the muscle proteins are partially denatured. As suggested by the name, the affected meat has a lighter colour, and its ability to bind water decreases (it contains less protein and more thiol groups and disulfide bridges). The fluid is released from the tissue and may drip from the surface, which leads to weight loss during storage and thawing of frozen meat.

In the DFD and DCB meats, the acidification 1 hour post-mortem results in a pH around 6.5 instead of 5.8 (the pH 24 hours post-mortem is about 6.3), which is explained by the loss of lactic acid in the blood while bleeding the animal. The result is meat with a high water binding capacity, but the meat consistency is stiff and the meat is dark. An additional problem with this type of meat is that it is more susceptible to spoiling since it lacks lactic acid, which normally impedes the growth of microorganisms after slaughter.

Appropriate handling of animals before and during slaughter can greatly reduce their discomfort and stress. This includes proper feeding and rest, as well as the use of suitable techniques for moving and transporting animals.

Thermal processing

During heat processing of meat, the first conformation changes to proteins occur at temperatures around 35 °C, when proteins in the sarcoplasm associate into unstable structures, reducing the water binding capacity of the meat and increasing its rigidity. The first visible changes occur at around 45 °C as a result of shortening of the muscles caused by myosin denaturation. At 50–55 °C actomyosin denatures, and between 55 and 65 °C the sarcoplasmic proteins denature (including globins bound to myoglobin and haemoglobin). Depending on the intensity and duration of the thermal processing, more severe changes in the structure of haematin occur (the central iron atom splits off and the porphyrin skeleton is degraded). As a result, the red colour of meat changes to reddish–brown and finally to grey–brown. Myoglobin is often stabilised by adding sodium nitrite to many meat products (see Section 9.2.1.5.3).

Denaturation of actomyosin and sarcoplasmatic proteins creates stable associated structures and a firm, solid gel (partial denaturation may also occur during local overheating, such as during meat grinding). Changes to the colour of meat are caused by oxidation of haem to haematin. The coagulation of proteins reaches a maximum at temperatures of 60–65 °C, which is followed by conformational changes (shrinkage to about a third or a quarter of the original length) of the collagen molecules (for fish this occurs even at 45 °C). At temperatures above 80 °C, virtually all myofibrillar and sarcoplasmic proteins are coagulated, and free thiol groups of actomyosin are oxidised to disulfide groups. Around 90 °C, collagen gelatinises and the water binding capacity of meat increases. At temperatures around 100 °C (during boiling of meat), there are also some chemical changes to the protein molecules. Significant reactions include desulfuration and deamination of proteins, yielding hydrogen sulfide and ammonia, which have an important impact on the development of the aromatic and taste properties of meat. These reactions are accompanied by a certain loss of cysteine/cysine and lysine.

Thermal processing of foods at temperatures around 150 °C (e.g. during baking) is much more complex than the reactions at 100 °C. The result is a certain loss of all amino acids, but also the formation of typical flavour substances. Processing at temperatures around 200 °C and higher in the surface layers of roasted, fried and grilled meat leads, in a greater extent, to the isomerisation of native L-amino acids bound in proteins to the corresponding D-amino acids and to less usual cross-links, such as lysinoalanine (see **2-121**). Some amino acids (such as tryptophan) and other nitrogenous compounds (such as creatinine) can produce toxic products (see Section 12.2.1.1), which are present at significant concentrations in grilled meat.

Drying meat and meat products (such as durable salami) leads to a significant aggregation of actomyosin molecules that partially denature, if they have not previously been denatured by heat, and the toughness of the meat or meat products increases. Freezing meat also induces significant changes in the structure of proteins. Myosin gradually denatures and associates with actin. This greatly strengthens the structure and decreases the water binding capacity of meat. However, these reactions do not significantly affect the nutritional value of meat. The texture and nutritional value of long-term stored frozen meat is, however, considerably influenced by reactions of proteins with oxidised lipids. Significant changes to proteins occur in the surface layers of smoked meat due to reactions with reactive aldehydes, such as formaldehyde and glyoxal, which form cross-links between polypeptide chains. Smoked products (e.g. meat and sausages) thus obtain a smooth and rigid surface.

2.4.5.2 Milk and dairy products

The main component of milk is water. Its content can vary considerably, according to the origin. Cow's milk contains 87–91% water. The basic chemical composition of cows', goat's and sheep milk in comparison with human milk is shown in Table 2.18. The amino acid composition of milk is given in Table 2.10.

Milk is a very complicated dispersed system (see Section 7.6.3.2.1) in which casein molecules form a micellar dispersion, globular whey proteins a colloidal dispersion, fat present in the form of fat droplets (milk microsomes) forms an emulsion, particles of lipoproteins form a colloidal suspension and low molecular weight substances (lactose and other sugars, amino acids, minerals and water soluble vitamins) form true solutions. Milk's typical white colour is related to the scattering and absorption of light by the fat particles and casein micelles. The yellowish colour that is sometimes seen is caused by carotenoid compounds dissolved in the fat phase (cream and butter), while the greenish colour of whey is caused by riboflavin.

The protein composition of cows' milk is shown in Table 2.19. Milk proteins are a mixture of two main types of proteins:

- **caseins** (approximately 80% of milk proteins)
- **whey** (**serum**) **proteins** (about 20% of milk proteins).

Table 2.18 Nutrient contents of milk.

Component	Content (%)			
	Cow's milk	Goat's milk	Sheep milk	Human milk
Total protein	3.2	3.2	4.6	0.9[a]
Caseins	2.6	2.6	3.9	0.4
Whey protein	0.6	0.6	0.7	0.5
Fat	3.9	4.5	7.2	4.5
Saccharides	4.6	4.3	4.8	7.1
Minerals	0.7	0.8	0.9	0.2

[a] The protein content increases during lactation to 1.6%.

Table 2.19 Protein composition of cow's milk.

Protein	Proportion (%)	Content (g/l)	Protein	Proportion (%)	Content (g/l)
Total caseins	80	25.6	***Total whey proteins***	20	6.4
α_S-Casein	42	13.4	α-Lactalbumin	4	1.3
β-Casein	25	8.0	Serum albumin	1	0.3
γ-Casein	4	1.3	β-Lactoglobulin	9	2.9
κ-Casein	9	2.9	Immunoglobulins	2	0.6
			Polypeptides (proteoses, peptones)	4	1.3

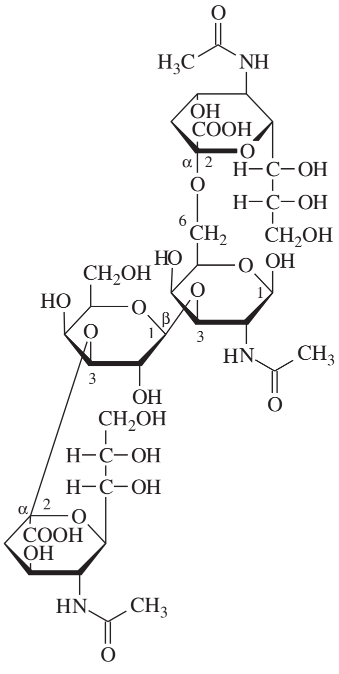
2.4.5.2.1 Caseins

The main components of the casein fraction in bovine milk are α_S-caseins. Bovine milk contains phosphoprotein α_{S1}-casein and α_{S2}-casein, both occurring in four genetic variants, identified by the letters A, B, C and D, which differ somewhat in their primary structure. The most common variant is B, which is a polypeptide chain composed of 199 amino acids (molecular weight 23.6 kDa, pI = 4.92–5.35). α_S-Caseins comprise from 44 to 55% of the total caseins and α_{S1}-casein comprises approximately 80% of the total α_S-caseins. The polypeptide chain contains eight phosphoserine residues located mainly at positions 43–80, which makes this part of the molecule polar. Non-polar side chains of amino acids are located in positions 100–199. α_{S1}-Casein forms insoluble calcium salts in the presence of calcium ions. Fragments of α_{S1}-casein are considered to be λ-casein. α_{S2}-Casein has a similar structure (molecular weight 25.2 kDa), but is less sensitive to the presence of calcium ions.

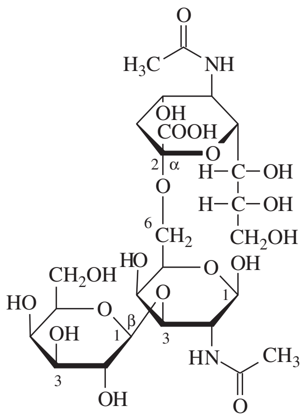
β-Casein comprises from 25 to 35% of the total casein. Polypeptide chains of β-casein consist of 209 amino acid residues (molecular weight 24 kDa, pI = 5.20–5.85). Each variant fits into one of two main categories known as A1 and A2. In cattle, A1-type β-caseins have the amino acid histidine at position 67, whereas the A2-type β-caseins have the amino acid proline at this position. They are also phosphoproteins, as they contain five phosphoserine residues (in positions 1–40). Non-polar side chains of amino acids are concentrated in positions 136–209. β-Caseins form soluble salts with calcium ions at temperatures of 1 °C and lower, which is insoluble at higher temperatures. Human β-casein is the major protein found in human milk.

The group of caseins (amounting to 3–7%) were in the past known as γ-caseins. γ-Caseins (pI = 5.8–6.0) are degradation products of β-caseins as a result of the action of indigenous milk proteinase (plasmin). Plasmin causes hydrolysis of β-casein, especially in late lactation and prior to milking. Splitting off the residue with amino acids 1–28 yields γ_1-casein (with a molecular weight of 20.5 kDa) and proteose-peptone-labeled PP8F, γ_2-casein (with a molecular weight of 11.8 kDa) is created by splitting off the amino acid residues 1–105 and γ_3-casein (with a molecular weight of 11.6 kDa) is formed by splitting off the amino acid residues 1–107.

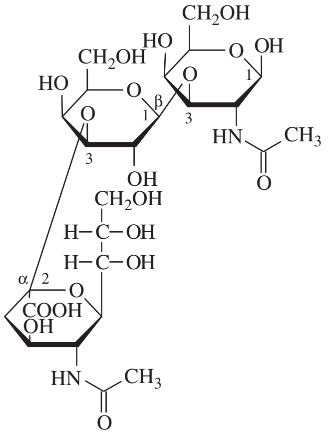
κ-Caseins occur in two genetic variants (A and B) in cow's milk, which represents about 8–15% of caseins. The molecule of the more common variant B is composed of 169 amino acid residues (molecular weight 18 kDa, pI = 5.37). The molecules occur as trimers and higher oligomers connected by disulfide bridges. Unlike the previous caseins α and β, κ-caseins are glycoproteins containing bound oligosaccharides composed of D-galactopyranose (D-Gal*p*), *N*-acetyl-D-galactosamine (D-Gal*p*NAc) and *N*-acetylneuraminic acid (NeuAc). The main component of κ-caseins (56.0%) is a branched tetrasaccharide (**2-99**), κ-caseins coupled with branched trisaccharides (18.5%, **2-100**), linear trisaccharides (18.4%, **2-101**), disaccharides (6.3%, **2-102**) and *N*-acetyl-D-galactosamine (0.8%) are found in smaller amounts. Sugars are bound to the protein via threonine at position 133 of the peptide chain through the glycosidic bond of *N*-acetyl-β-D-galactosamine (**2-103**). Calcium ions form soluble salts with κ-caseins that stabilise α_{S1}- and β-caseins in milk.



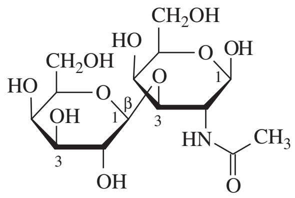
2-99, α-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(6→2)-α-NeuAc



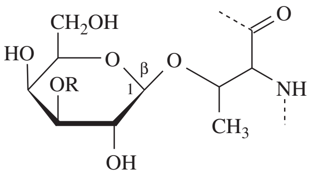
2-100, β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(6→2)-α-NeuAc



2-101, α-NeuAc-(2→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc



2-102, β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc



2-103, β-D-galactosamine residue in κ-caseins bound to threonine

Caseins in milk do not occur as monomers, but are aggregated into casein complexes and spherical particles called **micelles** (see Section 7.6.3.2.1). Caseins β and γ can easily associate into polymeric structures at about 20 °C, while at temperatures <8 °C they dissociate back to the monomers. The aggregation of molecules of α_S-, β- and κ-caseins into micelles occurs at temperatures >5 °C. Molecules of α_S-, β- and κ-caseins are first arranged into particles called **submicelles**, which have the shape of a rotating ellipsoid, and contain 25–30 protein molecules (Figure 2.25). The non-polar parts of molecules of caseins are oriented to the submicelle centre and bound through hydrophobic interactions. The polar molecules of caseins, that is, phosphoserine residues of α_S- and β-caseins and the threonine residue with bound oligosaccharides, interact with calcium ions and water. The individual submicelles are connected to each other via phosphate (phosphoserine) groups of α_S- and β-caseins (the κ-casein molecule does not have a bonding region) and calcium ions, either directly or through free phosphates and citrates (Figure 2.26).

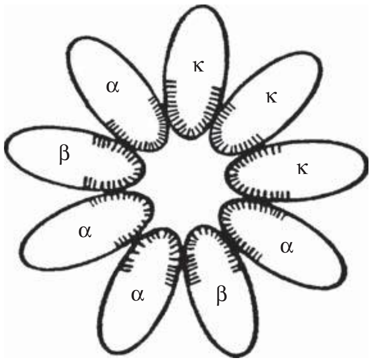


Figure 2.25 Cross section of a typical submicelle (hydrophobic parts of molecule are indicated by dashes). Coultate T.P (1989) p. 144, Reproduced by permission of the Royal Society of Chemistry.

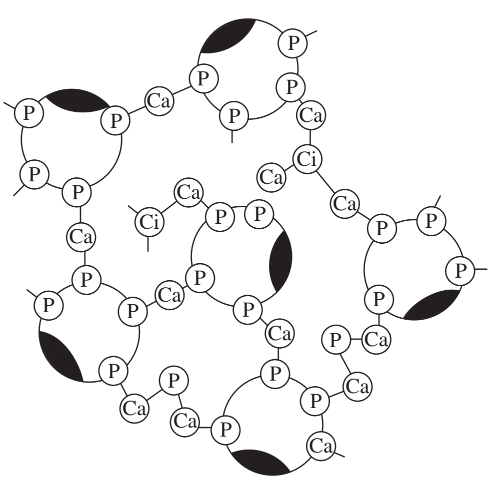


Figure 2.26 Interconnection of submicelles through phosphate (P), calcium ions (Ca) and citrate (Ci) (whole of the non-bonding areas with molecules of κ-casein are indicated). Coultate T.P (1989) p. 144. Reproduced by permission of the Royal Society of Chemistry.

A typical micelle of cows' milk contains approximately 20 000 casein molecules. About 93% (by weight of the micelle) are caseins, about 3% calcium ions, 3% inorganic (free) phosphates, 2% phosphates bound to phosphoserine, 0.4% citrates and 0.5% sodium, potassium and magnesium ions. The micelle diameter is 50–300 nm (the average is generally 150 nm). Their size depends on the α_S- and κ-caseins content. The smallest particles contain about 50% of

α-casein and 15% of κ-casein, while the largest particles contain about 42% α-casein, 26% κ-casein and about 30% β-casein. Milk contains about 1.10^{12} micelles in 1 ml.

2.4.5.2.2 Whey proteins

The globular protein β-lactoglobulin (Figure 2.21) constitutes about 50% of whey (serum) proteins. The relative molecular weight is 18 kDa and its polypeptide chain consists of 162 amino acids (pI = 5.35–5.41). β-Lactoglobulin occurs in three genetic variants, the main ones being labelled A and B. In milk it is present as a dimer that denatures irreversibly on heating (also in the presence of high concentrations of calcium ions in solutions of pH > 8.6). Thermally partially denatured protein reacts with other milk proteins (κ-casein and α-lactalbumin) through one accessible thiol group to form dimers linked by disulfide bonds.

Immunoglobulins, which are globular glycoproteins, are biologically important proteins with antibody efficacy, occurring in whey at low concentrations. They include IgG_1 (162 kDa, pI = 5.5–6.8), IgG2 (152 kDa, pI = 7.5–8.3), IgA (400 kDa as a dimer) and IgM (950 kDa as a pentamer). The clustering of fat globules in raw milk, which results in the formation of larger particles and finally in the formation of a layer of cream on the surface of the milk, is not caused by coalescence of globules, but is due to the action of a specific minor protein, **macroglobulin**, which creates cross-links between the globule membranes. Heating for several minutes to a temperature higher than 100 °C causes coagulation of this protein and prevents formation of a cream layer on the surface of pasteurised and otherwise heat-treated milk. Another important whey protein is α-lactalbumin, which has a biological function as a component of some enzymes. This protein occurs in two genetic variants (A and B), with a relative molecular weight of 14 kDa (pI = 4.2–4.5) and constitutes about 30% of whey proteins. Whey albumin (serum albumin) is present in smaller amounts, with a molecular weight of 66 kDa (pI = 5.13). Peptides and low molecular weight proteins called peptoses and peptones have relative molecular weights of 4–41 kDa (pI = 3.3–3.7).

2.4.5.2.3 Changes during storage and processing

Changes in the composition, structure and properties of milk proteins (caseins and whey proteins) occur during pasteurisation, sterilisation and especially during fermentation, drying and thickening of milk and in cheese making.

Thermal processing

Particularly labile milk proteins are the whey protein immunoglobulins, serum albumin, β-lactoglobulin and α-lactalbumin. During milk pasteurisation[10] at a temperature of 72–74 °C (20–40 s), about 50–90% of whey proteins are denatured and most enzymes present are inactivated. At temperatures >75 °C, the disulfide bonds in whey protein molecules are partly reduced to sulfhydryl groups and hydrogen sulfide is eliminated (mainly from β-lactoglobulin). The partial degradation of methionine yields thiols and disulfides, which also contribute to the so-called cooked flavour of milk. This defect is most obvious immediately after heating, but dissipates within 1 or 2 days. Sterilisation at 140 °C (for 4 s) denatures 100% of the milk proteins. Denatured milk proteins have a slightly higher nutritional value compared with the proteins of raw milk. The denatured β-lactoglobulin in canned milk can interact with κ-casein, which results in increased viscosity and sometimes even flocculation of sterilised milk.

Caseins in pasteurised and sterilised milk do not denature, but thermal processes lead, to a certain extent, to their dephosphorylation (phosphoserine hydrolysis), to proteolysis and aggregation of molecules. Casein could be completely dephosphorylated by incubation at 37 °C with 1% sodium hydroxide for a period of 24 hours. Under extreme conditions, such as during heating of sodium caseinate (see the next section) at 120 °C for 1 hour, about 50% of phosphoproteins are dephosphorylated and after 5 hours of heating the dephosphorylation reaches 100% and the extent of the caseinate hydrolysis is from 10 to 20%.

Lactose is the main milk sugar, which reacts with whey proteins, leading to loss of lysine. This reaction (the Maillard reaction) is particularly intense during milk evaporation or drying (see Section 4.7.5.12.3).

Precipitation and proteolysis of caseins

The pH of fresh milk is 6.5–6.75. When the pH decreases to 4.6, due to lactic acid formation, caseins precipitate, leaving a yellowish solution of whey (serum). The acidification (fermentation of lactose to lactic acid) is not only the spontaneous activity of contaminating microorganisms during storage of milk, but also a result of the activity of a range of cultural microorganisms used in dairy industry.

Partial precipitation of caseins occurs in the production of yoghurt and cottage cheese using appropriate strains of bacteria (such as *Streptococcus thermophillus* and *Lactobacillus bulgaricus*). Yoghurt gets its characteristic gel texture as a result of associations of casein micelles.

In the production of some hard and semi-hard rennet cheeses (e.g. Edam, Gouda, Cheddar and so on), the action of various cultural bacteria belonging to the genera *Streptococcus* and *Lactobacillus* acidify milk to a pH of around 5.5, which partly transforms lactose into lactic acid. At this stage, the proteolytic enzyme rennet (also called rennin, but the term chymosin is preferred) is added, which accomplishes the coagulation of milk. Chymosin is extracted from the stomachs of calves, but today it is often replaced by proteases from fungi of the genus *Mucor* and by pepsin obtained from the stomachs of chickens or pigs. Chymosin specifically hydrolyses κ-casein almost exclusively at the phenylalanine (105)–methionine (106) bond (Figure 2.27), and the molecule splits into two parts:

- ***para*-κ-casein**
- **κ-casein macropeptide**.

[10] Pasteurisation, in order to extend shelf life and inactivate pathogenic microorganisms, is carried out at temperatures of 85 °C (2 s), 71–74 °C (14–40 s) or 62–65 °C (30 min). UHT (ultra high temperature) milk is obtained by indirect heating at temperatures of 135–140 °C (6–10 s) or by direct steam heating at 140–150 °C (2–4 s).

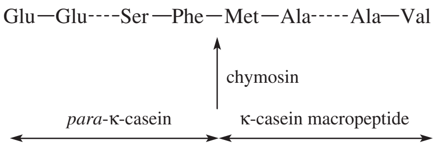


Figure 2.27 Hydrolysis of κ-casein by rennet.

The significance of the primary, secondary and tertiary structures of κ-casein on the sensitivity of this bond has now been established, as have pH, ionic strength, temperature, heat treatment of milk, environmental and processing variables. The resulting *para*-κ-casein (protein composed of 105 amino acids) contains the original hydrophobic part of the κ-casein molecule and, therefore, remains part of the casein micelles, but unlike native κ-casein it no longer has the micelle stabilising function. With the participation of Ca^{2+} ions, the bonds that develop between the micelles cause casein coagulation (curdling). κ-Casein macropeptide is a glycopeptide composed of 64 amino acid residues corresponding to the *C*-terminal 106–169 residues of κ-casein. Owing to the bound oligosaccharides, κ-casein macropeptide is hydrophilic and passes later into the whey.

After several hours of storage, the casein curds reach the desired firmness and are cut into small pieces, which causes the curds to shrink and allows the whey to be released. Heat drives the shrinking (syneresis) process even further. The increase in temperature causes the protein to shrink due to increased hydrophobic interactions and also increases the rate of fermentation of lactose to lactic acid. The increased acidity also contributes to shrinkage of the curd particles. When the curds have reached the desired moisture and acidity they are separated from the whey. Curd handling from this point on is very specific for each type of cheese. After salting (salting of brine is used in Gouda, salting in a vat in Cheddar and salting of the cheese surface in Feta production) and other operations (such as pressing and milling), the curds are left to mature (ripen) at low temperature until the characteristic flavour, body and texture profile is achieved. This final stage varies from weeks to years according to the particular cheese. During maturation, proteins are partly hydrolysed by chymosin and by microbial proteases and milk fat is partly hydrolysed by milk lipase. Proteolytic and lipolytic reactions are necessary to obtain the desired texture, taste and smell of the cheese. Proteolysis is probably the most important biochemical reaction during the ripening of most cheeses, having a major impact on their flavour and texture.

Use of whey and casein

Whey is the cloudy, yellowish liquid that is left after milk has curdled. Sweet whey (pH 6.5–6.7) is a by-product when milk is curdled with chymosin in cheese production. Acid or sour whey (pH 4.3–4.6) results from milk to which an acid has been added to aid in the curdling process (in the production of some types of Mozzarella cheeses). Whey has often been used to feed livestock, but the presence of proteins and other nutritionally important constituents led the food industry to take advantage of this in a wide range of whey and whey constituents applications. For example, acid whey is used as a raw material for the production of lactose and other sugars (see Section 4.4.3.1.2). Sweet whey is a raw material in the production of some cheeses (including the Italian cheese ricotta). Dried whey is added to some food products (infant formulae, breads, pancakes and biscuits) to enhance their nutritional value and functional properties. Whey protein concentrate is used to improve athletic performance and as an alternative to milk for people with lactose intolerance and so on.

About 50 years ago, the major uses of casein were in technogical applications, but nowadays casein products are regularly used as food additives, for example as ingredients that enhance some physical properties of foods, such as whipping, foaming, water binding, thickening and emulsification and nutritional properties.

Insoluble sweet casein (pH 6.5–6.7) obtained by precipitation of milk with chymosin is still used for technological purposes, for instance in the production of imitation tortoise shell and ivory (combs, hairclips, knife handles, piano keys and other products). The insoluble acid casein (pH 4.3–4.6), obtained by precipitation of milk using mineral acids or lactic acid, is a raw material for the production of caseinates. Caseinates are produced by reaction of acid casein curd or dry acid casein with dilute alkali. The resulting solution can be spray dried to produce a caseinate powder. The most common alkali used in the manufacture of spray dried sodium caseinate is sodium hydroxide, where a solution is mixed with a slurry of the casein powder in water. The pH of sodium caseinate ranges from 6.5 to 6.9. The manufacture of other caseinates is very similar to that of sodium caseinate. Caseinates are used in chemical and other industries. Soluble caseinates (sodium, potassium and ammonium caseinates; solubility 100%) and dispersible calcium (solubility 90–98%) and magnesium caseinates are used in various branches of the food industry as additives for binding water and emulsifying agents. For example, sodium caseinate is used as an emulsifying and foaming agent in the production of mayonnaise, ice creams, processed cheeses, coffee whiteners, instant breakfasts and beverages and meat products, potassium caseinate is used in the manufacture of confectionery, a mixture of sodium, potassium and calcium caseinates is employed in the production of confectionary products, to stabilise and fortify meat products, yoghurts and breads. Technological uses of acid casein include, for example, production of synthetic fibres, paints, adhesives for wood, paper and foil laminates, coatings for paper and cardboard.

The so-called insoluble co-precipitates contain all of the milk proteins (caseins and serum proteins). They are obtained from milk by precipitation with acids, the addition of salts and by heating. Like caseinates, co-precipitates are used as food additives for some meat products.

2.4.5.3 Eggs

The eggs of birds and reptiles have been eaten by mankind since time immemorial. The most commonly consumed eggs are those of hens,

Table 2.20 Average content of nutrients in hen egg.

Component	Content (%)		
	Shell	Egg white	Egg yolk
Proteins	6.4[a]	9.7-10.6	15.7-16.6
Fats	0.03	0.03	32.0-35.0
Carbohydrates	-	0.4-0.9	0.2-1.0
Minerals	91.9[b]	0.5-0.6	1.1
Water	1.6-1.7	88	49
Total weight in %	9.5[c]	63	27.5

[a]Complex of proteins with mucopolysaccharides (50:1).
[b]Inorganic salts: calcium carbonate (98.4%), magnesium carbonate (0.8%), tricalcium phosphate (0.8%).
[c]Including shell membrane.

ducks, geese, quails and ostriches.[11] Eggs contain a considerable amount of protein in a concentrated form (about 13% of the edible part), which has high nutritional value. The basic chemical composition of a hen's egg (average weight is around 58 g) is summarised in Table 2.20. The egg white proteins constitute about 53% and yolk proteins 47% of the total protein. The levels of the individual proteins in the egg white and yolk are shown in Table 2.21. The amino acid composition of egg proteins is shown in Table 2.11.

2.4.5.3.1 Egg white proteins

The egg white constitutes about two-thirds of the weight of a whole fresh hen's egg (minus its shell), and contains more than 40 different proteins, which are classified as globulins, glycoproteins and phosphoproteins. Glycoproteins contain various oligosaccharides composed of galactose (Gal), mannose (Man), acetylderivatives of glucosamine (Glc*N*Ac), galactosamine (Gal*N*Ac) and neuraminic acid (NeuAc) (Table 2.22).

The main protein of egg white is a group of related compounds known as ovalbumin A, or ovoalbumin A (relative molecular weight of 44.5 kDa, pI = 4.6–4.8; coagulation temperature ≥57.5 °C depending on pH). In addition to sugars, ovalbumin A contains two molecules of bound phosphoserine and four thiol groups (two of which form a disulfide bridge). During storage of eggs, reactions of thiol groups and of the disulfide group in ovalbumin A produce ovalbumin S, which is more thermoresistant and coagulates at 92.5 °C (in solutions of pH 7), but denatures relatively easily when the egg white is whipped. Of particular importance for the stability of whipped foam are ovoglobulins G_2 (40 kDa, pI = 5.5) and G_3 (58 kDa, pI = 4.8).

Ovotransferrin, also known as ovoconalbumin or conalbumin (76 kDa, pI = 6.1), which in hens is identical with serum transferrin, shows antimicrobial effects. This protein coagulates at a lower temperature than ovalbumin (coagulation temperature is 53 °C) and forms complexes with divalent and trivalent metal ions. Complexes with iron can cause a pink discolouration of products containing egg white.

Ovomucoid and ovomucin are the proteins responsible for the viscosity and gel-like consistency of egg white. Ovomucoid (28 kDa, pI = 4.4–4.6) is composed of three components which differ in carbohydrate composition on the *N*-terminus of the molecule. Ovomucoid inhibits trypsin and other proteolytic enzymes and is a strong allergen. The stability to thermal denaturation (in an acidic medium) is associated with nine disulfide bonds in the molecule.

[11]Also eaten, although rarely, are the eggs of some wild birds, such as those of lapwings (e.g. in Great Britain and the Netherlands). The large-scale collection of such eggs for food is now prohibited as it has contributed to a decline in the numbers of birds.

Table 2.21 Composition of proteins in the white and yolk of hen's eggs.

Proteins	Content		Proteins	Content	
	%	g/kg		%	g/kg
Total egg white proteins	100	106	***Total egg yolk proteins***	100	166
Ovalbumin	54	57	***Granules***	47	78
Ovotransferrin (conalbumin)	12	13	Lipovitellenins (I-VII)	37.3	62
Ovomucoid	11	12	Lipovitellin apoproteins (HDL)[a]	40	66
Globulin G_1 (lysozyme)	3.4	4	α-Lipovitellin		
Globulin G_2	4	4	β-Lipovitellin		
Globulin G_3	4	4	Phosvitin (phosphovitin)	13.4	22
Ovomucin	3.5	4	LDL[b]	12	20
Ovoflavoprotein	0.8	1	***Plasma***	53	88
Ovoglycoprotein	1.0	1	Lipovitellenin (LDL)[b]	16	27
Ovomacroglobulin (ovostatin)	0.5	1	Livetins	15	25
Ovoinhibitor	1.5	2	α-Livetin (serum albumin)		
Avidin	0.05	<1	. . . β-Livetin α_2-glycoprotein)		
Cystatin	0.05	<1	. . . γ-Livetin (γ-globulin)		

[a]Lipoprotein fractions (see Section 3.6.1) of high density (high density lipoprotein).
[b]Lipoprotein fractions of low density (low density lipoprotein).

Table 2.22 Composition of saccharides in important glycoproteins of egg white and yolk.

Protein	Content (%)	Content (mol/mol of protein)				
		Gal	Man	Glc*N*Ac	Gal*N*Ac	NeuAc
Ovalbumin	3	-	5	3	-	-
Ovotransferrin	-	-	4	8	-	-
Ovomucoid	23	2	7	23	-	1
α-Ovomucin[a]	13	21	46	63	6	7
Ovoglycoprotein	31	6	12	19	-	2
Ovoinhibitor A	9	10[b]	-	14	-	0.2
Avidin[c]	10	-	4 (5)	3	-	-
Phosvitin	-	3	3	5	-	2

[a]Contains sulfuric acid bound as ester.
[b]Sum of galactose and mannose content.
[c]Basic unit (16 kDa), Gal = D-galactose, Man = D-mannose, Glc*N*Ac = *N*-acetyl-D-glucosamine, Gal*N*Ac = *N*-acetyl-D-galactosamine, NeuAc = *N*-acetylneuraminic acid.

Ovomucin is also thermostable and exists in two forms differing in their content of *N*-acetylneuraminic acid (α-form 220 kDa, β-form 720 kDa, $pI = 4.5–5.0$).

The basic protein ovoglobulin G_1 (lysozyme;14.3 kDa, $pI = 10.7$) contains four disulfide bonds. It occurs in many animal tissues and secretions (e.g. tears), in the latex of some plants and in microorganisms. This protein possesses the activity of *N*-acetylmuramidase, hydrolyses the β-glycosidic linkage between *N*-acetylmuramic acid and *N*-acetyl glucosamine in the peptidoglycan murein, which is the construction material of cell walls in gram-positive bacteria (see Section 4.3.3.7). Lysozyme is therefore used as an antimicrobial agent.

Native ovoflavoprotein (49 kDa, $pI = 5.1$) has, as does ovomucoid, certain antinutritional effects, as it inhibits serine proteases (trypsin, chymotrypsin and also microbial proteases) and has antiviral activity. Ovomacroglobulin (ovostatin) is an inhibitor of serine, cysteine, thiol and metalloproteases and shows antimicrobial activity. Some antinutritional effects are also seen in the basic glycoprotein avidin in raw egg white (relative molecular weight of the monomer is 15.6 kDa). It contains four identical subunits ($pI = 9.5$), each of which binds one molecule of biotin to give an unavailable complex. However, the denatured avidin, for example in hard-boiled eggs, does not interact with biotin. The interaction of riboflavin with flavoprotein (32 kDa, $pI = 4.0$) has, on the contrary, a positive influence on vitamin stability. Cystatin acts as cysteine protease inhibitor, and shows antimicrobial, antitumor and immunomodulating activities.

2.4.5.3.2 Egg yolk proteins

The egg yolk is an emulsion of fat in water, which makes up about 36% of the weight of a whole fresh hen's egg. The dry matter consists of roughly one-third protein and two-thirds lipid. Egg yolk contains a range of droplets (20–40 μm in diameter), similar to fat droplets, which are enveloped by a lipoprotein membrane composed mainly of LDL lipoproteins (Table 2.21). It also contains granules (1–1.3 μm in diameter) consisting of proteins, lipids, minerals and a plasma. Egg yolk proteins are various glycoproteins, lipoproteins, glycophosphoproteins and glycophospholipoproteins. The major granule proteins are lipovitellin and phosvitin, and lipovitellenin and livetin are mainly present in the plasma.

Granule proteins

The most important lipoprotein (HDL) is lipovitellin, which contains neutral triacylglycerols (35%), polar phospholipids (about 60%) along with cholesterol and its esters (5%). α-Lipovitellin and β-lipovitellin differ in their phosphorus content. In the yolk, lipovitellin forms complexes with phosvitin. This name includes two aggregates of related molecules, α-phosvitin (160 kDa) and β-phosvitin (190 kDa). α-Phosvitin is composed of three basic units (37.5, 42.5 and 45 kDa, respectively) while β-phosvitin has only one unit (45 kDa). Phosvitin is glycophosphoprotein (Table 2.22) with a high content of phosphoric acid (10%) bound in the form of phosphoserine, which provides efficient metal-binding sites for calcium, iron and other cations. This sequestering activity of phosvitin is related to its biological functions (the availability of metal ions for the developing embryo).

Plasma proteins

The name lipovitellenin refers to lipoproteins of the LDL type, where the lipid portion represents approximately 84–90% of the molecule weight. This lipid portion is composed of triacylglycerols (74%) and phospholipids (26%). Livetins are the water-soluble fraction of globular proteins, and includes α-livetin (albumin), β-livetin (glycoprotein), γ-livetin (globulin) and δ-livetin (polypeptide). These proteins are identical to serum proteins of hens, namely serum albumin, α_S-glycoprotein and γ-globulin, respectively. Immunoglobulin IgY (167 Da, $pI = 5.7–7.6$) represents the main antibody of egg plasma and blood serum, and is analogous to mammalian immunoglobulin G (IgG). It is transmitted from the blood into the yolk and contributes to the development of immunity in the chicken in its early stages of life. The isolated protein is used in human and veterinary medicine.

2.4.5.3.3 Changes during storage and processing

Eggs are usually eaten fresh or refrigerated. Further industrial processing produces a variety of egg products, such as pasteurised, frozen or dried egg whites, egg yolks or the whole egg contents. Smell and taste defects affect the long-term storage of raw eggs. Sulfur and nitrogen compounds (such as hydrogen sulfide, thiols, sulfides and amines) arising from cysteine and methionine and indoles derived from tryptophan are particularly responsible for these changes.

The mechanical denaturation of proteins occurs, for example, when whipping egg whites. Denatured proteins located at the interface with the air have a positive effect on foam stability. During the heat treatment of eggs, white protein denaturation begins at temperatures around 57 °C. In the temperature range 60–65 °C,

most egg white proteins are denatured (with the exception of ovomucoid, ovomucin and complexes of avidin with biotin) and at temperatures of 65–70 °C most of the yolk protein (except for phosvitin) is denatured. Egg proteins play a major role in the development of the typical aroma of boiled and fried eggs. When drying egg whites, yolks or whole egg contents, glucose in egg white reacts with lysine bound in proteins and with phosphatidylethanolamine bound in egg yolk lipoproteins, which leads to undesirable colour and flavour changes in the product. Before drying, glucose in egg whites is therefore removed by fermentation using bacteria of the genera *Aerobacter* or *Streptococcus* or the *Saccharomyces cerevisiae* yeasts. Another possibility is oxidation of glucose to gluconic acid using the oxidoreductase glucose oxidase (in the presence of catalase). Cold storage of pasteurised egg whites slightly increases the viscosity of the material, but the functional properties remain practically unchanged. For yolks and whole egg contents, cold storage leads to a substantial increase in viscosity due to the formation of products of a gel consistency. Slow freezing (critical temperature is –6 °C) leads to changes to the conformation of the lipoprotein complex and probably irreversible partial dehydration of the protein portion of the lipoproteins.

2.4.5.4 Foods of plant origin

Plant protein sources provide 65% of the world's supply of protein, with cereal grains (47%) and pulses, nuts and oilseeds (8%) as the other major sources. Of the cereals, wheat (43%), rice (39%) and maize (12%) are the main contributors. Other limited sources of plant protein are fruits, leaves, tubers and other parts of plants included under the terms fruits, vegetables or root crops. Plant protein sources can differ from animal protein sources in terms of digestibility, amino acid composition, the presence of antinutritional (such as enzyme inhibitors) and toxic factors (e.g. saponins, cyanogens and lectins), which adversely influence protein digestibility, nutritional value and food safety.

The amino acid composition of cereal grains and other seeds is different to that in foods of animal origin. Seed proteins usually contain a large amount of aspartic and glutamic acids and their amides, but some essential amino acids occur in lower levels and become the limiting amino acids. With some exceptions (such as soybeans), lysine levels are uniformly lower in wheat, rice, maize, potatoes and other crops. Soybeans have lower levels of the sulfur amino acids, there is a lower level of tryptophan in maize, and a lower level of threonine in cassava. As a consequence, the nutritional value of plant protein is lower than that of animal protein; nevertheless, plants can cover all human protein needs. An appropriate combination of plant materials gives a mixture of proteins with high nutritional value, which can provide the protein for the diet of vegetarians. However, the question is whether this is only achievable with the carefully selected diets consumed by vegetarians, or whether it could also be achievable with the cereal or other staple diets available to poor or developing communities. In addition to protein, the body receives a number of other nutritionally valuable components such as fibre, carbohydrates, vitamins and minerals from plants.

2.4.5.4.1 Cereals and pseudocereals

The most important sources of vegetable protein for human nutrition are cereal grains, primarily wheat, which is the third most produced cereal after maize and rice. The protein content of the sub-aleurone layer of wheat, located between the white centre of the grain and the brown fibrous outer layer, is higher than in the central part. Therefore, the protein content in flour significantly depends on the degree of milling (flour extraction rate) and, of course, on the plant species, variety and other factors. Dark wholemeal flour has a higher protein content than white flour; the difference is about 4%. The basic chemical composition of common cereals and pseudocereals is shown in Table 2.23. The basic proteins of cereals are albumins, globulins, prolamins and glutelins. Their trivial

Table 2.23 Average basic chemical composition of cereals and pseudocereals (%).

Cereals[a]	Water	Proteins	Total lipids	Total sugars	Starch	Total dietary fiber	Minerals (ash)
Wheat	12.1	12.1	2.0	0.4	64.0	12.5	1.5
Rye	12.2	11.0	1.7	1.0	52.4	15.1	1.9
Barley	10.5	11.6	2.2	0.8	52.2	17.3	2.3
Oats	10.6	14.8	6.3	-	40.1	10.6	2.9
Rice (brown)	11.8	7.7	2.7	0.9	70.4	3.5	1.5
Rice (white)	13.3	6.5	0.5	-	72.5	2.8	1.2
Maize	11.5	9.3	4.3	0.6	62.6	7.3	1.3
Millet (pearl)	8.7	11.0	4.2	-	70.0	8.5	1.8
Buckwheat	9.8	13.3	3.4	-	70.0	10.0	1.9
Amaranth	11.3	13.6	7.0	1.7	50.0	6.7	2.1

[a] Average values from various sources, therefore their sum may exceed 100%. Non-starch carbohydrates are calculated by difference.

names are based on the Latin names of the plants they come from (see Section 2.4.1).

Wheat proteins

Wheat usually contains 7–13% protein, but can contain up to 15%, with lysine as the limiting amino acid. Distribution of proteins within the wheat kernel differs significantly. About 72% of total protein is in the endosperm, 15.5% in the aleurone layer, 4.5% in the pericarp, 4.5% in the scutellum and 3.5% in the embryo, while starch only occurs in the endosperm. The major factors influencing the protein content of wheat are genetic (species and variety) and environmental conditions (timing and amount of growing season precipitation, temperature during the growing season, soil nitrogen reserve levels and applied nitrogen fertilisers). Wheat proteins are composed of albumins known as leucosin (about 14%), globulins known as edestin (8%), prolamins known as gliadin (33%) and glutelins known as glutenin (46%). In other words, about 20–25% of wheat proteins are represented by water-soluble cytoplasmic proteins that predominantly have structural, metabolic and protective functions (e.g. enzymes with activity of α- and β-amylases, proteases, lipases, phytases and lipoxygenases) and 75–80% of proteins are water-insoluble storage proteins, prolamins and glutelins. The total number of wheat proteins is about 40.

The reserve, water-insoluble proteins are the major proteins of wheat. Prolamins (gliadins) and glutelins (glutenins) are represented by a number of related proteins, each with slightly differing amino acid compositions (there are several dozen gliadins in each of the wheat varieties). Gliadins have a relative molecular weight of between 30 to more than 100 kDa (usually between 60 and 80 kDa). Three basic fractions are identified: high molecular weight gliadins (relative molecular weight is >100 kDa), ω-gliadins (60–80 kDa) and low molecular weight gliadins (30–40 kDa). The ω-gliadin fraction contains the so-called α-, β- and γ-gliadins. Gliadins contain a large amount of glutamine (36–45%), proline (14–30%), somewhat less aspartic and glutamic acids and unusually low amounts of the basic amino acids arginine, lysine and histidine. The relatively low content of acidic and basic amino acids with polar side chains is linked to low gliadin solubility. Glutenins have a higher relative molecular weight, which typically ranges from 40 to 20 000 kDa, (usually about 2000 kDa), as they are made up of polypeptide chains linked by disulfide bridges.

The high proline content virtually prevents the formation of helical secondary structures in both types of proteins. Gliadin and glutenin molecules therefore contain only short helical sections associated with segments of the unorganised secondary structure. The ω-gliadin molecules (stabilised by strong hydrophobic interactions) are rich in segments known as β-turns. Their number increases with rising temperature. In contrast, the α-, β- and γ-gliadins contain α-helices (30–35%) and β-sheets (about 10% in α-gliadins) stabilised by disulfide bridges and hydrogen bonds. Heating causes an increase in the unorganised structures and partial loss of the α-helical structures.

Bread flour is obtained from wheat varieties with higher protein content (12–14%) and is also known as strong flour. This term relates to the properties of the dough, which is elastic and stiffer and therefore requires more intense mixing, retains the carbon dioxide produced by yeast and air well and provides voluminous products. The milling and baking properties of flour are linked not only to the protein content, but also to its composition. Flours referred to as weak flours usually contain less than 10% protein. They are suitable for the production of biscuits, pastries, confectionery and other products (along with baking powder), but not for making bread.

Wheat flour gives a dough with water, which is basically (in addition to starch) a viscoelastic sticky material composed of two-thirds water and one-third hydrated gliadins and glutenins, **gluten**. The formation of the large quaternary structure of gluten during dough-mixing and bread-making processes is extremely complex and not well characterised. It has been established that the glutenin subunits, directly affects dough formation and bread-making quality, the typical viscoelastic properties of gluten, as their molecules are able to generate a three-dimensional network stabilised by different types of bonds between the glutenin molecules. The aggregated glutenin polymers range in relative molecular weight from about 1 000 000 to 10 000 000 Da and consist of three, four or five glutenin subunits, together with an uncertain number of subunits of low molecular weight. Hydrogen bonds are important for their stabilisation and are mediated mainly by glutamine residues, ionic and hydrophobic interactions of amino acids, as well as disulfide bonds present in the gluten structure that contribute to the process of dough formation through a disulfide/sulfhydryl exchange. Tyrosine bonds (bityrosine derivatives) formed in wheat dough during the processes of mixing and baking also contribute to the structure of the gluten network (see Section 2.5.1.1.3). The main factor that determines the quality of flour is the ratio between glutenins and gliadins. Gliadin molecules have a modifying effect on the viscoelastic properties of dough. Gluten proteins in some individuals cause an autoimmune disorder of the small intestine called celiac disease (see Section 10.3.1.1.2). The amino acid composition of wheat protein can be seen in Table 2.12.

Proteins of other cereals and pseudocereals

Rye albumins (44%) and globulins (10%), representing about 55% of rye proteins, have similar properties to albumins and globulins of wheat. About 45% of rye proteins are prolamins, known as secalin (21%), and glutelins, known as secalinin (25%). The trivial names of insoluble proteins of rye and other grains are seldom used, but have become known by the collective name gluten, as in the case of wheat proteins. However, this so-called rye gluten differs from wheat gluten in the proportion of prolamins and glutelins, the content of some amino acids and, especially, in its viscoelastic properties. Wheat gluten is ductile, while rye gluten tears, but rye is still the only cereal grain, in addition to wheat, from which a typical bread can be produced. Nevertheless, rye bread has a completely different texture and consistency (it is compact and sticky) from wheat bread, and also a different content of other flour components, in particular pentosans (see Section 4.5.1.5.2).

The main components of barley and oat proteins are prolamins (hordein in barley, 25%; gliadin in oats, 15%) and glutelins (hordenin in barley, 55%; avenin in oats, 54%), albumins (12%

in barley, 20% in oats) and globulins (8% in barley and 12% in oats known as avenalin), which are minor proteins. Important constituents of barley albumins are the lipid transfer protein (about 9 kDa; responsible for the shuttling of phospholipids and other fatty acid esters between cell membranes) and protein Z (43 kDa; a major barley endosperm albumin) that survive the malting and brewing processes and play roles as foam-promoting agents during these processes. Rice proteins are mainly composed of glutelins known as oryzenin (about 80%), while prolamins known as oryzin (2%) and other proteins (albumins 11% and globulins 10%) are minor components. Maize proteins are composed of small amounts of albumins (4%), globulins (3%), gliadins called zein (about 50%) and glutelins (20–45%). The typical limiting amino acids in maize gluten are lysine and tryptophan.

Increasingly, previously little known or almost forgotten grains are appearing in human nutrition, such as millet, sorghum and the pseudocereals, which include buckwheat and amaranth. Some cereals with increased protein content have also been newly introduced. An example might be the interspecies hybrid of wheat and rye called triticale (the name comes from Latin names *Triticum* for wheat and *Secale* for rye). The grains contain 15–20% protein. The protein content in the interspecies hybrid of wheat and barley, which is called tritordeum (the Latin name for barley is *Hordeum*), is 19–22%. The amino acid composition of the major cereals and pseudocereals is shown in Table 2.12.

2.4.5.4.2 Legumes and oilseeds

Legumes and oilseeds are important sources of proteins, lipids and other nutrients in the diet. Legumes are plants of the Fabaceae family that are cultivated for their seeds used for human and animal consumption and as forage crops (e.g. alfalfa and clover). The most important edible legume seeds (pulses) are soybeans, peas, beans, lentils and peanuts, in addition to some other relatively less frequently used legumes (e.g. chickpeas, vigna and lupin seeds). Legumes are high in protein (a typical protein content is 20–45%) and the limiting amino acids are usually sulfur amino acids.

Proteins in soybeans, peas and other legumes are mostly composed of globulins, while minor components are low molecular weight albumins. For example, soybean protein contains about 80% globulins. Globulins in many legumes are further divided into legumins and vitilins, but soybean globulins are known as glycinin and conglycinin, respectively. Legumins have higher relative molecular weight, are less soluble and have better thermal stability than vitilins. Peanuts contain about the same amount of globulin arachin and related conarachins, 15% of albumins and 10% of glutelins. Pea protein contains about 70% globulins (legumin and vicilin), 20% albumins and 10% glutelins. Recent, more precise characterisation of legume proteins is based on their sedimentation coefficients during ultracentrifugation. In this way, soybean proteins can be separated into four fractions designated 2S, 7S, 11S and 15S.[12] The 2S fraction termed α-conglycinin contains 8–22% of soybean protein and consists of a number of enzymes (the Bowman–Birk and Kunitz trypsine inhibitors and cytochrome c). The 7S globulin fraction, known as β- and γ-conglycinins, accounts for 35–37% of the total protein (containing haemaglutinin, lipoxygenase, β-amylase and globulins labelled a, a_1 and b). The 11S fraction that accounts for 31–52% of the total protein is known as glycinin, and the 15S fraction, which has no trivial name, accounts for 5–11% of the total protein and is poorly characterised. It is thought to be composed of polymers of other soy proteins.

The typical basic chemical composition of some legumes and oilseeds is summarised in Table 2.24. The content of the individual nutrients and other components, however, varies broadly and depends on the seed origin (plant variety), maturity and many other factors. For example, the protein content of common mature pea seeds varies between 14.2 and 36.1% and dietary fibre content is 16.7–25.5% and water content is 11%. Green peas, however, contain 78.9% water, 5.4% protein and 5.1% dietary fibre. Furthermore, oilseeds accumulate oil rather than starch. The amino acid composition of important legumes is given in Table 2.24.

Some legume seeds that contain significant amount of lipids, such as soybeans and peanuts, are used as oilseeds. The protein content of oilseeds usually ranges from 20 to 50%. Other important oilseeds include rapeseed, sunflower seed and many other plant

[12] S denotes the so-called Svedberg's units in which the sedimentation rate is expressed. It is equal to 1×10^{-13} s.

Table 2.24 Average basic chemical composition of some legumes and oilseeds (%).

Mature seeds[a]	Water	Proteins	Total lipids	Total sugars	Starch	Total dietary fibre	Minerals (ash)
Soybeans	8.5	40.2	20.5	7.3	0.5–2.0	9.3	4.5
Peanuts	6.5	27.6	49.6	4.0	1.0	6.0	2.8
Peas	11.3	26.3	2.2	15.8	40	15.9	2.9
Beans (kidney)	11.8	27.2	1.0	2.1	40	11.9	3.8
Chickpeas	11.5	20.7	5.7	10.7	47	13.9	3.6
Lentils	10.4	27.7	2.1	2.0	44	12.2	2.9

[a] Average values from various sources, therefore their sum may exceed 100%. Non-starch carbohydrates are calculated by difference.

seeds described in Section 3.4.3.4.1. Almonds and nuts have high protein contents (about 20%). Owing to their low consumption, these commodities cannot be considered significant sources of protein. The amino acid composition of major oil seeds and nuts is given in Table 2.13.

However, legumes and oilseeds often contain various antinutritional factors or even toxic substances. Examples of such substances are different allergens, protease inhibitors, lectins, saponins, indigestible sugars such as raffinose and its homologues, phytic acid and others (see Section 10.3.2.6).

2.4.5.4.3 Changes during storage and processing

Denaturation of proteins can occur during grain drying and milling, but on a very small scale. The extent of protein denaturation depends on water activity, temperature and time of drying and milling. When completely denatured, gluten lacks its typical viscoelastic properties. Bread flour improves its baking properties during storage of up to about a year, but with longer storage these properties deteriorate. The improved baking properties of flour are caused by natural bleaching, which is based on autoxidation of unsaturated fatty acids (see Section 3.8.1.8.2) and their enzymatic oxidation by lipoxygenases. The resulting hydroperoxides of fatty acids are strong oxidising agents that oxidise the free thiol groups of gluten proteins. Their polypeptide chains are connected by disulfide bridges to high-molecular weight units, which have a positive effect on the volume of a loaf and the texture of the crust. At the same time, degradation of carotenoid pigments (see Section 9.9.5.1) results in a more attractive flour of lighter colour. Bread flour is sometimes conditioned with ascorbic acid, which increases the bread volume and creates a better texture. The mechanism of this reaction is described in Section 5.14.6.1.6. Attempts to simulate the aging process has led to the use of flour additives (oxidising agents) in some countries, such as chlorine dioxide, dibenzoyl peroxide, bromates and iodates of alkali metals (see Section 11.4.2.2.1, Section 5.2.6.2 and Section 5.14.4.4).

During the mixing of wheat flour with water, yeast and other ingredients, the dough is worked mechanically (the process is known as kneading). Flour proteins gliadin and glutenin are hydrated, and air and carbon dioxide (produced by yeast) are incorporated, which is the basis for the structure of the bread. On baking, water evaporates from the crust, the starch begins to gelatinise at about 50 °C, and proteins are denatured at about 70 °C, releasing water that is absorbed by the starch and which increases the loaf volume. As the temperature increases, the loaf shape is fixed, and gluten creates the typical three-dimensional net structure, which surrounds all the other ingredients. The colour of the crust begin to develop. Baking bread and other cereal products results in a relatively large Maillard reaction, which is desirable in order to develop the typical colour and flavour. However, it has a negative effect on the nutritional value of proteins, because the bound essential amino acid lysine primarily enters this reaction and thus becomes unavailable (blocked).

Significant changes to the structure of proteins also occur during other methods of thermal processing of cereals. For example, extrusion leads to texturised proteins that lose their prevailing globular structure, they gain a fibrous structure and are denatured, which is accompanied by the disruption of existing non-covalent interactions and disulfide bridges, and the emergence of new bonds between filamentous molecules.

2.4.5.5 Non-traditional protein sources

Since the middle of the last century, unicellular and multicellular organisms have been studied as possible non-traditional sources of protein for human nutrition and livestock. Special interest has been paid to yeast proteins (e.g. the genera *Candida* or *Torula*), and also to bacteria, fungi and algae (e.g. the green sweet water algae of the genus *Chlorella*, which contain about 45% protein dry matter) and to higher plants (oilseed meals, the leaves of certain plants and some vegetables and foliage). These protein sources have not found significant use in human nutrition, but are often used as animal feed.

However, various technologies have been developed that utilise extractions from oilseed meals and pulses as the raw material for the production of flours, protein isolates and protein concentrates. These technologies are mainly used in the processing of soybeans and, to a lesser extent, in the processing of peanuts, cotton, lupine and other oilseed meals. Additional sources of protein are whey, fishmeal and others. The final products can be various mixtures rich in proteins (often enriched by minerals and vitamins), which are mainly used in less-developed countries.

Protein concentrates generally tend to contain around 50% protein, although a higher protein content (90%) is found in **protein isolates**. Concentrates are mostly prepared from oilseed meals (such as soybean meals). The proteins are first denatured by heating, and soluble substances are extracted with water. Protein isolates are prepared by extracting proteins and other substances soluble in dilute aqueous solutions of sodium or calcium hydroxides. The extract obtained is purified and its pH is adjusted to the value of the isoelectric point, when the proteins precipitate. This procedure gives a relatively clean proteinaceous material, which can then be used as a dietary supplement or as an intermediate for further processing. The texture of protein preparations can be further modified to resemble meat. Well known examples are so-called soy meat, soy curd (tofu) or soy cheese (sufu). Modifications include addition of fat, flavouring agents, dyes and binders, and the finished products are mostly processed by extrusion.

2.4.5.6 Modified proteins

The modification of native protein structure is based on the nutritional, hygienic and toxicological and technological requirements. This can be carried out chemically or enzymatically, with the aim of improving:

- desirable physico-chemical properties of proteins (solubility, dispersibility, elasticity, viscosity, adhesivity, cohesivity, the ability to bind water, form gels, emulsions and foams and stabilise these dispersion systems)

- nutritional value (by inactivation of antinutritional and toxic substances, through improved availability of essential amino acid content)

- their organoleptic properties (texture and taste)

- the use of non-traditional raw materials for food purposes (e.g. yeast proteins).

Functional groups of bound amino acids can be chemically modified in various ways. Amino groups can be derived by acylation or methylation (reaction with formaldehyde and reduction of the resulting hydroxymethyl derivatives). Carboxyl and hydroxyl groups can be derived by esterification, amide bonds (including peptide bonds) by hydrolysis, thiol groups by oxidation to disulfides and vice versa. For example, succinylated casein is, unlike native caseins, practically insoluble in solutions of pH 2–3 and soluble in solutions of pH 4.5. Succinylated yeast protein is soluble in solutions of pH 4–6, is more resistant to heat denaturation and has a higher emulsifying strength. Alkylated proteins, for example, do not react with reducing sugars in the Maillard reaction. The enzyme modifications are mainly based on the protein dephosphorylation and plastein reaction (see Section 2.3.2.2). For example, in the presence of calcium ions, the dephosphorylated β-casein is more soluble than the native protein. The plastein reaction has found use in the debittering of protein hydrolysates and for the incorporation of nutritionally valuable essential amino acids into proteins of low nutritional value (e.g. tryptophan, threonine and lysine into maize zein).

2.5 Reactions

Biochemical (enzymatic) reactions *in vivo* transform free and bound amino acids into a wide range of products that can be involved in the biosynthesis of many secondary metabolites and affect various biological processes. In food raw materials, which mainly include animal tissues (meat, fish and poultry) post-mortem and plant tissues during post-harvest storage, a variety of biochemical and chemical reactions take place that involve free amino acids and amino acids bound in proteins and peptides and which influence the nutritional, sensory, technological and hygienic and toxicological quality of foods. Other reactions of these compounds are caused by the action of various physical factors (such as heat, mechanical force, hydrostatic pressure and radiation) and chemical agents (acids, bases, salts and surfactants) during processing and cooking. These reactions often result in oxidation of side chains, peptide chain fragmentation, aggregation, enzymatic inactivation and conformational changes known as denaturation (changes to the native conformation stabilised by non-bonding interactions) and even in changes to the primary structure of proteins due to hydrolysis of peptide bonds. Proteins, peptides and amino acids react with each other and with other natural food components, particularly with oxidised lipids, reducing sugars in the Maillard reaction, oxidised phenolic compounds, some food additives (acids and alkalis, nitrites and sulfites), oxidising agents and some contaminants (e.g. with halogenated solvents). The extent and type of reactions that take place during food storage and processing depend on the particular food (its chemical composition), conditions during storage and processing (water activity, temperature, pH, oxygen and other factors), and the reaction partners present.

The positive consequences of these changes, interactions and reactions are the inactivation of undesirable enzymes and microorganisms, denaturation of protein antinutritional factors and toxins, which results generally in higher digestibility, production of desired flavour-active compounds, desired colour changes and increased food shelf life. The negative consequence is some reduction of the nutritional value of the food (reduced amounts of certain essential amino acids, reduced digestibility of proteins and reduced utilisation of some reaction products). Sometimes, the formation of undesirable flavour compounds and undesirable discoloration may also occur. Some reactions may also negatively affect the hygienic–toxicological quality of food products, because some reaction products have specific biological effects and are classified as technological (process-induced) contaminants (see Section 12.2).

2.5.1 Intramolecular and intermolecular reactions

Amino acids in living organisms undergo oxidation during normal biosynthesis when a diet is rich in protein and during starvation, which contributes significantly to the generation of energy. In animal tissues (meat, fish and poultry) post-mortem and plant tissues during post-harvest storage, amino acids undergo enzymatic and chemical oxidation along with other reactions, such as decarboxylation, transamination, oxidative decarboxylation and reactions with other food components (lipids, sugars and their decomposition products), which produce a variety of products.

2.5.1.1 Oxidation

Proteins and free amino acids contain numerous reactive functional groups and can become substrates for oxidoreductases or may react with reactive oxygen species, such as hydroxyl radicals ($HO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), fatty acid hydroperoxides (R–O–OH), alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals, atmospheric oxygen and other oxidising agents, often under the catalysis of transition metal ions.

Oxidation of the polypeptide chain of proteins can lead to various di(oligo)mers or hydroxy derivatives. The reaction of proteins with the most reactive oxygen species, hydroxyl radicals, results in abstraction of the hydrogen atom from the α-carbon, from the polypeptide chain and also from the side chains of hydrophobic amino acids residues (Figure 2.28). The reaction is analogous to the autoxidation of fatty acids (see Section 3.8.1.8.2). Abstraction of a hydrogen atom by a hydroxyl radical yields a carbon radical, which reacts with oxygen with the formation of a hydroperoxyl radical. This radical is stabilised by reaction with a hydrogen donor (and also with a protonated superoxide radical or Fe^{2+} and H^+ ions),

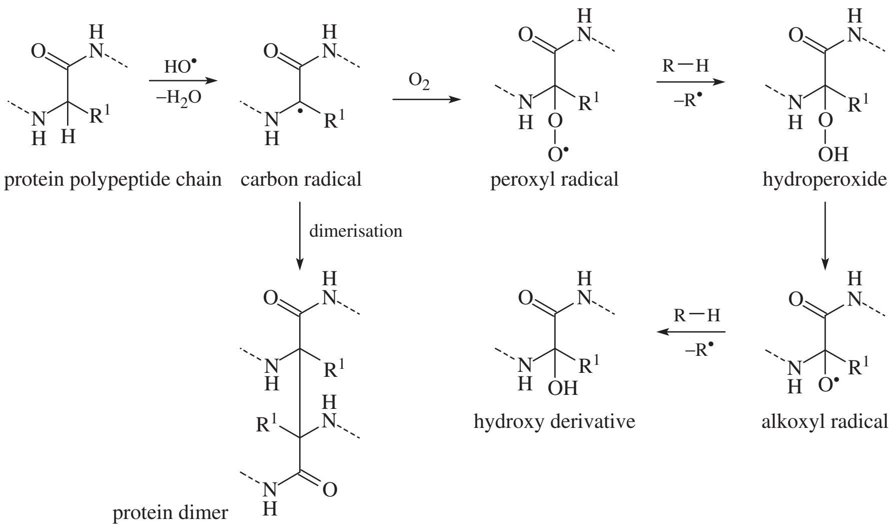


Figure 2.28 Oxidation of protein polypeptide chain by reactive oxygen species.

which yields unstable hydroperoxide that is decomposed to an alkoxyl radical (in the presence of a protonated superoxide radical or Fe^{2+} ions). Dimerisation of the carbon radical in the absence of oxygen produces a protein dimer and the reaction of the alkoxyl radical with a hydrogen donor (protonated superoxide radical or Fe^{3+} and H^+ ions) gives a hydroxylated protein.

Free peroxyl radicals react with proteins with the formation of protein free radicals, which react with other protein free radicals to yield protein dimers, or with lipid free radicals to yield copolymers. The occurrence of a lipoprotein with covalent bonds and protein oligomers during oxidation of proteins (PH) by alkoxyl ($RO^\bullet$) and peroxyl ($ROO^\bullet$) radicals is shown in the following equations. A protein radical ($P^\bullet$) forms most frequently by splitting off the labile hydrogen atom on C_α, an alkoxyl radical yields a hydroxy acid and a peroxyl radical yields hydroperoxide:

$$\text{P–H} + \text{R–O}^\bullet \rightarrow \text{P}^\bullet + \text{R–OH}$$
$$\text{P–H} + \text{R–O–O}^\bullet \rightarrow \text{P}^\bullet + \text{R–O–OH}$$

Recombination of protein radicals subsequently yields protein oligomers:

$$\text{P}^\bullet + \text{P} \rightarrow \text{P–P}^\bullet$$
$$\text{P–P}^\bullet + \text{P} \rightarrow \text{P–P–P}^\bullet \text{ etc.}$$

$$\text{P}^\bullet + \text{P}^\bullet \rightarrow \text{P–P}$$
$$\text{P–P}^\bullet + \text{P}^\bullet \rightarrow \text{P–P–P etc.}$$

Cross links of the lipid–protein type are produced mostly by the following reactions:

$$\text{P}^\bullet + \text{R–O–O}^\bullet \rightarrow \text{P–R} + \text{O}_2$$
$$\text{P}^\bullet + \text{R} - \text{O}^\bullet \rightarrow \text{P–O–R}$$
$$\text{P}^\bullet + \text{R}^\bullet \rightarrow \text{P–R}$$

Oxidation of the polypeptide chain of proteins can also lead to the cleavage of peptide bonds. Two different mechanisms are proposed, α-amidation and diamide pathways (Figure 2.29). In both pathways, the polypeptide chain is shortened and the *N*- and *C*-amino acid residues of the resulting fragments are modified (aspartyl, glutamyl and prolyl residue produce specific *N*- and *C*-terminal fragments).

Sulfur-containing amino acids (cysteine and methionine), aromatic and heterocyclic amino acids (phenylalanine, tyrosine, histidine and tryptophan) are relatively oxylabile compounds. In addition to these amino acids, the side chains of some proteins are also readily oxidised. Some oxidation products are not available, as the source of the amino acids and some products are undesirable

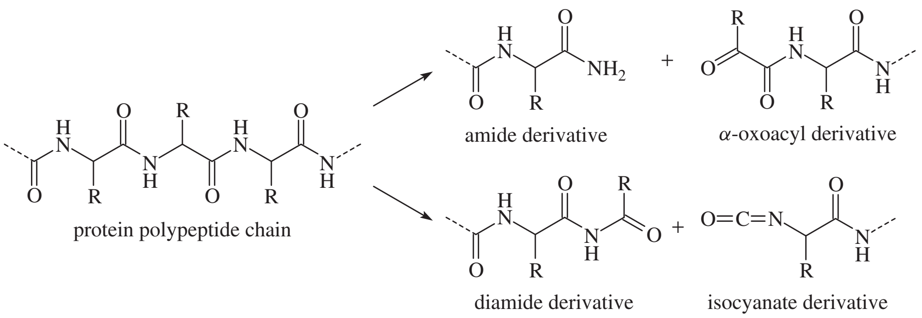


Figure 2.29 Oxidative cleavage of protein polypeptide chain. Stadtman and Levine 2003. With kind permission from Springer Science and Business Media.

as off-flavours and because of hygienic and toxicological aspects. Oxidation of proteins resulting from oxidative stress, which modifies their structure and affects their function, is related to organism aging and pathogenic changes in some diseases.

2.5.1.1.1 Cysteine and cystine

Oxidation of the thiol group (–SH) to the disulfide group (–S–S–) proceeds typically through the action of dehydrogenases, reactive oxygen species (such as singlet oxygen), fatty acid hydroperoxides and even by atmospheric oxygen (autoxidation). Cysteine (free or bound in peptides and proteins) yields cystine (Figure 2.30). The reaction is reversible and the cleavage of the disulfide bond in cystine may proceed, according to the conditions, by homolytic (photolysis and other reactions) or heterolytic mechanisms.

It is assumed that the first stage of cysteine autoxidation by homolytic cleavage is the formation of the alkylthiolate (RS^- anion) through the reaction of the thiol with a hydroxyl ion (reaction therefore takes place faster in alkaline media). Alkylthiolates react with oxygen to form a thiyl radical ($RS^•$) and anion of superoxide radical ($O_2^{-•}$) (see Section 3.8.1.13.2). Homolytic cleavage of thiols by reactive oxygen species also produces thiyl radicals (Figure 2.31). Volatile thiols, which are components of many food flavours, are oxidised in the same way (see Section 8.2.9.1.2).

Cysteine is oxidised to cystine more effectively by fatty acid hydroperoxides, hydrogen peroxide and reactive oxygen species. Fatty acid hydroperoxides simultaneously oxidise sulfur atoms and cystine yields thiosulfinates (monooxides) and also products containing two sulfoxide groups (disulfoxides), a sulfonic group (dioxide or thiosulfonate), a sulfoxide and sulfone group (sulfoxidosulfones or trioxides), or two sulfonic groups (disulfones or tetraoxides; Figure 2.32). In foods, the degree of oxidation does not usually exceed the level of thiosulfinate and dioxide. These compounds are the main products of cysteine and cystine oxidation. Their bioavailablity is about 20–50% that of cysteine. The product of cysteine oxidation by fatty acids hydroperoxides (and hydrogen peroxide) is, in addition to cystine, the unstable cysteine sulfenic acid, cysteine sulfinic acid and cysteine sulfonic acid known as cysteic acid (Figure 2.33). Both of the latter acids are completely unavailable as a source of cysteine.

The glutathione sulfhydryl groups are oxidised to disulfide groups or can be converted into the corresponding sulfenic, sulfinic and sulfonic acids.

$$2\,R{-}SH \xrightleftharpoons{1/2\,O_2,\ -H_2O} R{-}S{-}S{-}R$$

Figure 2.30 Oxidation of cysteine to cystine, R = $CH_2CH(NH_2)COOH$.

$$R\text{-}SH + HO^- \rightleftharpoons R\text{-}S^- + H_2O$$
$$R\text{-}S^- + O_2 \rightleftharpoons R\text{-}S^• + O_2^{-•}$$
$$2\,R\text{-}S^• \longrightarrow R\text{-}S\text{-}S\text{-}R$$
$$R\text{-}SH + O_2^{-•} \longrightarrow R\text{-}S^• + HO_2^-$$
$$R\text{-}SH + HO^• \longrightarrow R\text{-}S^• + H_2O$$

Figure 2.31 Oxidation of cysteine, R = $CH_2CH(NH_2)COOH$.

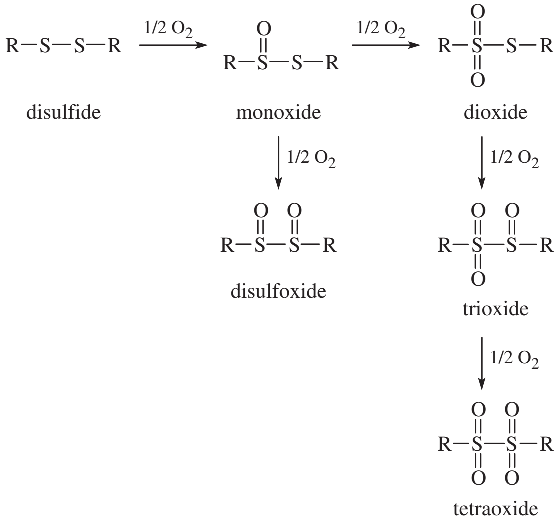


Figure 2.32 Oxidation of cystine, R = $CH_2CH(NH_2)COOH$.

$$\underset{\text{thiol}}{R{-}SH} \xrightarrow{1/2\,O_2} \underset{\text{sulfenic acid}}{R{-}SOH} \xrightarrow{1/2\,O_2} \underset{\text{sulfinic acid}}{R{-}SO_2H} \xrightarrow{1/2\,O_2} \underset{\text{sulfonic acid}}{R{-}SO_3H}$$

Figure 2.33 Oxidation of cysteine to acids, R = $CH_2CH(NH_2)COOH$.

2.5.1.1.2 Methionine

Methionine can be oxidised by reactive oxygen species, fatty acid hydroperoxides and oxidised polyphenols. The primary oxidation product, methionine sulfoxide (free or bound in proteins), is a frequent component of various proteins. The contents of methionine sulfoxide in protein sources used for foods and feeds usually vary from 0 to 30% of the total methionine content (e.g. up to 11% methionine sulfoxide apparently occurs in milk powder, 13% in lean beef, 16–28% in wheat gluten and 50% in orange juice). It is fully available as a source of methionine as it can be reduced back to methionine by sulfoxide reductases, but the final oxidation product, methionine sulfone, is unavailable as a source of methionine (Figure 2.34). The oxidation of the methyl group to

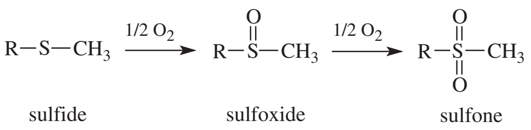
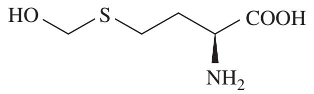


Figure 2.34 Oxidation of methionine, R = $CH_2CH_2CH(NH_2)COOH$.

a hydroxymethyl group by hydroxyl radicals gives L-2-amino-4-(hydroxymethylthio)butanoic acid (**2-104**). The so-called hydroxymethionine, (*R*,*S*)-2-hydroxy-4-(methylthio)butanoic acid, and its salts are used in cosmetic preparations intended to prevent the signs of the cutaneous ageing, such as sagging of the cutaneous and subcutaneous tissue and loss of cutaneous elasticity.



2-104, L-2-amino-4-(hydroxymethylthio) butanoic acid

2.5.1.1.3 Phenylalanine and tyrosine

Phenylalanine residues in proteins and free phenylalanine are oxidised by various reactive oxygen species to 2- and 3-hydroxy derivatives, and possibly other products too.

The most important reaction is the direct post-translational enzymatic oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) in animals *in vivo*, which is catalysed by tyrosine hydroxylase. Subsequent reactions then lead to melanins, an important group of pigments widely distributed in all living organisms (see Section 9.3.1.1). Tyrosine is also a substrate of oxidoreductases in the enzymatic browning reactions in foods (see Section 9.12).

Oxidation catalysed by peroxidases (Figure 2.35) converts the tyrosine residues in proteins into bityrosine and isobityrosine derivatives. Bityrosine has been found in animal proteins from resilin, a protein found in the cuticle of insects and arthropods, to elastin and collagen in vertebrates. Both bityrosine and isobityrosine, as well as some higher order structures, have been shown to be synthesised in human phagocytes by a myeloperoxidase–hydrogen peroxide system. Bityrosine, formed by oxidation of tyrosine residues with reactive oxygen species and oxidising agents (ascorbic acid, azodicarbonamide and potassium bromate) was also discovered in wheat flour, dough and bread. It has been suggested that it is a stabilising cross-link in the wheat gluten structure in addition to disulfide bonds. Singlet oxygen mediated oxidation of tyrosine residues in proteins suggests the initial formation of an unstable endoperoxide via 1,4-cycloaddition of singlet oxygen to the aromatic ring. The endoperoxide undergoes ring opening to produce a hydroperoxide that decomposes to the corresponding alcohol. The hydroperoxide derived from free tyrosine yields a hydroperoxide of an indole derivative by ring closure, which is transformed to the corresponding alcohol and other heterocyclic products (Figure 2.36).

2.5.1.1.4 Histidine

Oxidation of histidine by singlet oxygen results in a complex mixture of products. The initial 1,4-cycloaddition of singlet oxygen yielding one or more endoperoxides is followed by subsequent ring opening, which produces unstable hydroperoxides decomposing to a variety of products via free radicals. Some intermediates can form adducts with various nucleophilic reagents, dimers with another histidine molecules and a range of other products. The final reaction products are aspartic acid, asparagine and urea. The reaction

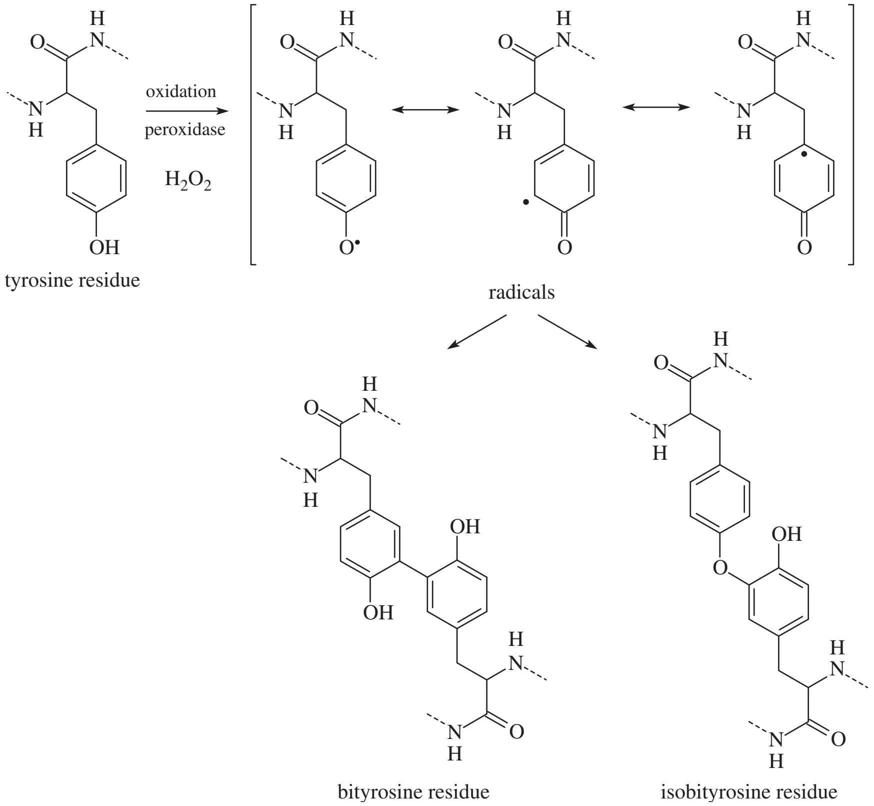


Figure 2.35 Oxidation of tyrosine by peroxidases.

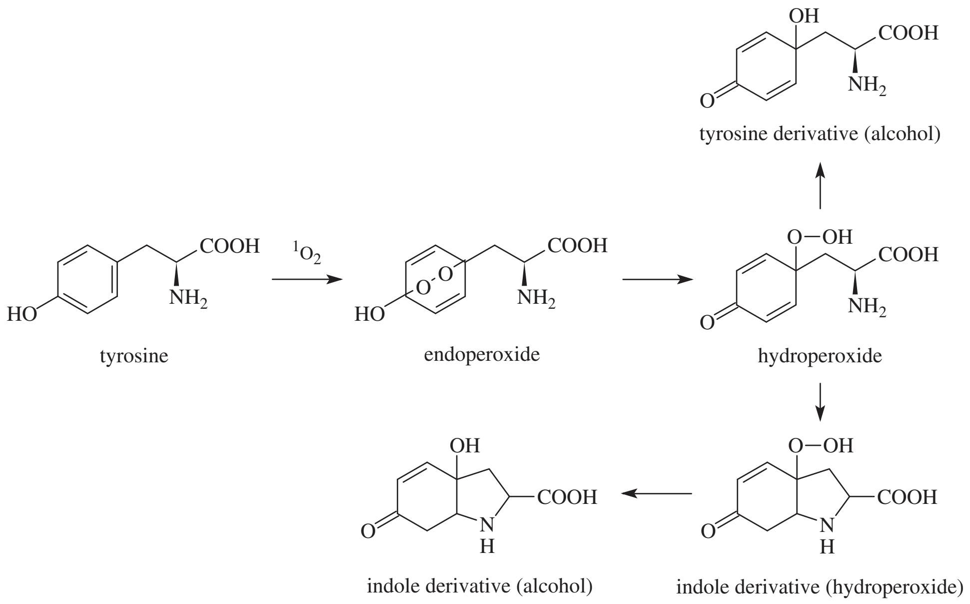


Figure 2.36 Oxidation of tyrosine by singlet oxygen. Wright, Bubb, Hawkins and Davies, 2002, Scheme 1. Reproduced by permission of John Wiley and Sons.

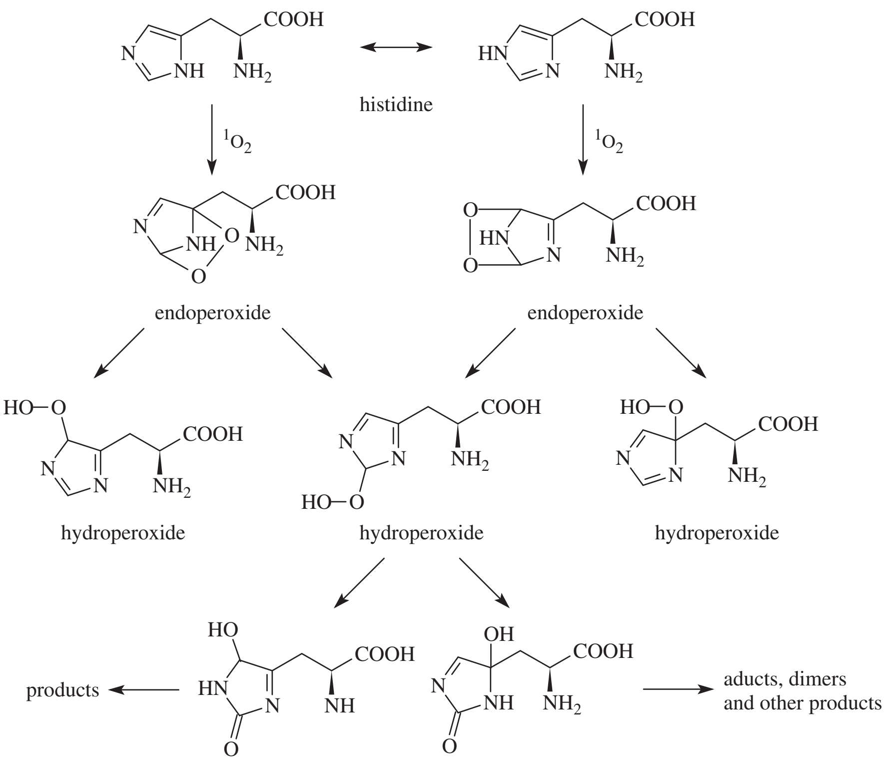
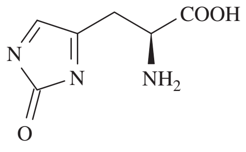


Figure 2.37 Oxidation of histidine by singlet oxygen. Agon, Bubb, Wright, Hawkins and Davies (2006). Reproduced by permission of Elsevier.

is schematically illustrated in Figure 2.37. Recent studies revealed the formation of 2-oxohistidine (**2-105**) in the histidine residues in proteins. The oxo group can be hydrated or undergo reaction with a lysine side chain amine group to form a protein cross-link.



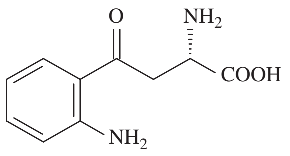
2-105, 2-oxohistidine

2.5.1.1.5 Tryptophan

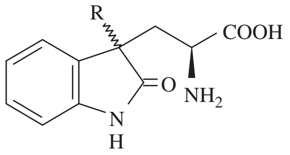
Tryptophan (free and bound in proteins) is a very oxylabile compound, especially in acid solutions. Free tryptophan and tryptophan residues in proteins are oxidised by reactive oxygen species, by hydrogen peroxide, fatty acid hydroperoxides, sulfoxides, peroxyacids and other oxidation agents and can also react via physical quenching, which results in energy transfer and de-excitation of singlet oxygen.

The main products of tryptophan oxidation with singlet oxygen are a dioxethane derivative, formed via 1,4-cycloaddition and a hydroperoxide at C-3. Subsequent decomposition of these intermediates yields *N*-formylkynurenine, whereas their ring closure leads to *cis*- and *trans*-isomers of 3α-hydroxypyrroloindoles, that is, 3a-hydroxy-1,2,3,3,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acids and 3α,8α-dihydroxypyrroloindoles. The simplified reaction sequences are given in Figure 2.38.

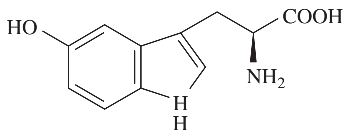
The oxidation of tryptophan with hydrogen peroxide yields a number of oxygen-containing products, via *cis*- and *trans*-isomers of hydroperoxides and free radicals. The main degradation products are the normal physiological metabolites *N*-formyl-L-kynurenine and L-kynurenine (3-anthraniloyl-L-alanine, **2-106**), totally unavailable as a source of tryptophan. Other reaction products include 3α-hydroxypyrroloindole derivative (Figure 2.38), 2,3-dihydro-2-oxotryptophan or 2-hydroxytryptophan (keto–enol tautomerism), also known as oxindolylalanine (**2-107**), dioxindolylalanine (**2-107**), that is, 2,3-dihydro-3-hydroxy-2-oxo-tryptophan and 5-hydroxytryptophan (**2-108**).



2-106, kynurenine, R = H



2-107, oxindolylalanine, R = H
dioxindolylalanine, R = OH



2-108, 5-hydroxytryptophan

The main intermediate in tryptophan degradation occurring in acid hydrolysates of proteins, where tryptophan is completely degraded, is oxindolylalanine (**2-107**), followed by simple indoles, such as 3-hydroxy-2-oxindole (**2-109**) and 2-oxindole (**2-109**). The reaction of tryptophan with cystine in acid solutions yields cysteine and α-amino-2-[(2-amino-2-carboxyethyl)thio]-1*H*-indole-3-propionic acid, known as tryptathionine (**2-110**). The tryptathionine cross-link formed between tryptophan and cysteine is characteristic of the toxic bicyclic peptides amatoxins and phallotoxins (see Section 10.3.2.9.2). 2-Aminoacetophenone (**2-111**) (together with its *N*-formyl derivative) is known as the character impact compound responsible for the atypical gluey and glutinous off-flavour of milk powders and sulfurated wines, which is a measure that is indispensable for white wine making. In non-fat

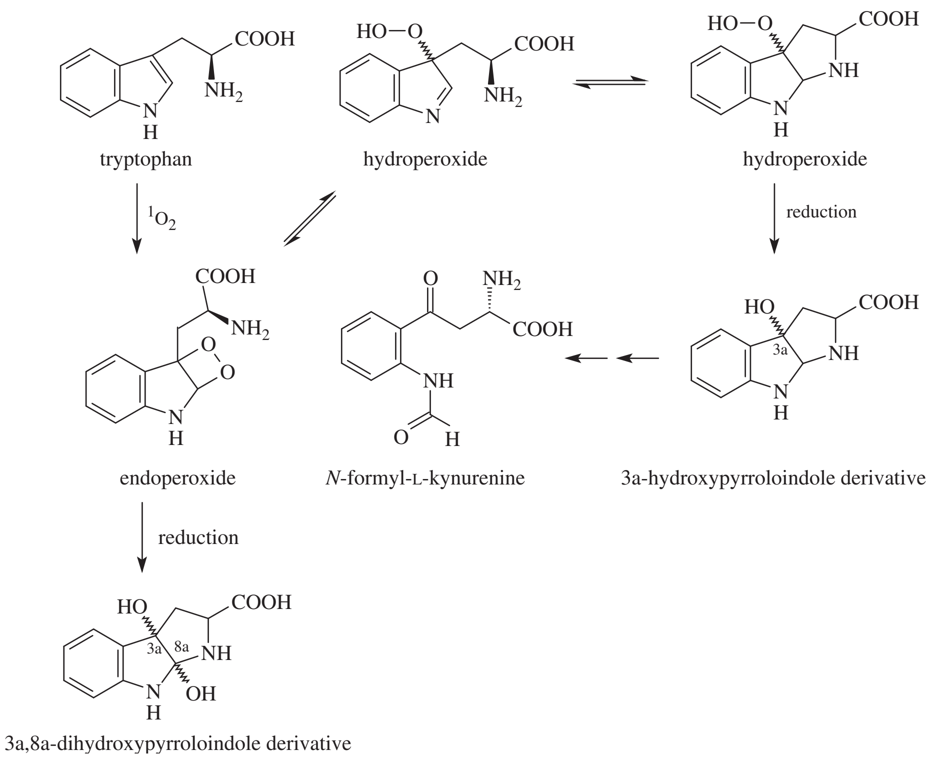
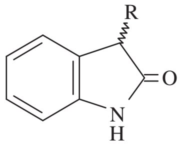
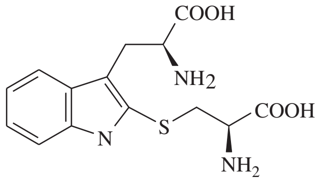


Figure 2.38 Oxidation of tryptophan by singlet oxygen. Gracanin, Hawkins, Pattison and Davies (2009). Reproduced by permission of Elsevier.

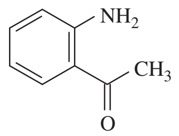
dry milk it is responsible for a strong flavour characterised as potato-like or cocoa-like. In wine, where it is formed by oxidative degradation of the phytohormone indole-3-acetic acid triggered by sulfuration, its flavour resembles naphthalene. Its odour threshold is 1 μg/l in wine. The products of microbial degradation of tryptophan, indole (**2-112**) and 3-methylindole known as skatole (**2-112**), are responsible for the off-flavour of some foods, such as stored eggs and boiled tripe (see Section 8.2.11.1.4).



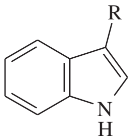
2-109, 2-oxindole, R = H
3-hydroxy-2-oxoindole, R = OH



2-110, tryptathionine



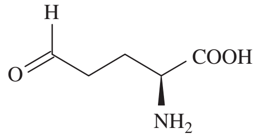
2-111, 2-aminoacetophenone



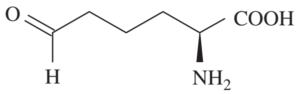
2-112, indole, R = H
skatole, R = CH_3

2.5.1.1.6 Other amino acids

In addition to the side chain of bound cysteine that is oxidised to bound cystine, other amino acid side chains in proteins can also react with reactive oxygen species (such as hydroxyl radicals) with catalysis by transition metal ions, which leads to generation of hydroxyl, oxo and formyl protein derivatives or peptide fragments. For example, the side chain of bound leucine is oxidised to 3-, 4- or 5-hydroxyleucine, the hydroxyl group of threonine is oxidised to an oxo group (yielding 2-amino-3-oxobutanoic acid on hydrolysis), the side chain of arginine yields a glutamic acid 5-semialdehyde residue (**2-113**), while the side chain of lysine produces 2-aminoadipic acid 6-semialdehyde residues (**2-114**).



2-113, glutamic acid 5-semialdehyde



2-114, 2-aminoadipic acid 6-semialdehyde

2.5.1.2 Isomerisation

Conventional processing of food raw materials, as well as culinary methods used during food preparation, does not usually cause a significant isomerisation (racemisation) of L-amino acids (Table 2.25) (with the exception of aspartic acid). L-Aspartic acid and L-serine undergo racemisation relatively easily. L-Isoleucine (isomerises to D-*allo*-isoleucine), L-proline, L-threonine (isomerises to D-*allo*-threonine) and L-valine yield smaller amounts of D-isomers. Free amino acids are roughly ten times more stable than amino acids bound in proteins. An extensive racemisation of amino acids does however occur, even at relatively low temperatures, in alkaline media used to inactivate enzymes, microorganisms, microbial

Table 2.25 D-Amino acids contents of some foods.

Food	Content of D-amino acids as a % of total amount of both optical isomers							
	Ala	Asp	Glu	Leu	Met	Phe	Pro	Val
Hamburger (outer layer)	2.8	5.5	2.4	3.2	2.9	2.7	1.8	1.5
Original beef meat	3.2	6.2	4.9	3.1	2.4	2.8	2.0	1.6
Pasteurised milk	1.8	7.3	5.1	-	-	-	-	-
Condensed milk	2.5	-	-	-	-	-	-	-
Original milk	1.9	7.3	4.8	-	-	-	-	-
Sour milk[a]	38.6	14.1	4.0	18.4	-	-	-	6.8
Kefir[a]	37.4	17.6	4.9	22.6	-	-	-	5.6
Yoghurt[a]	61.3	20.9	12.4		-	-	-	-
Toast (outer layer)	2.8	10.5	3.2	2.7	1.7	2.4	2.1	1.1
Originál bread (white)	2.4	5.6	2.8	3.2	2.3	2.3	0.9	0.9
Extruded soybean flour	2.7	7.6	3.9	2.7		2.4	1.6	0.8
Original soybean flour	2.5	4.4	3.1	1.4		2.8	2.3	1.0
Roasted almonds	7.2	4.7		6.5		1.3		1.1
Original almonds	0.0	2.6		3.0		1.1		0.0

[a] Amino acids are mainly formed by hydrolysis of peptidoglycans of microbial cell walls that contain D-amino acids.

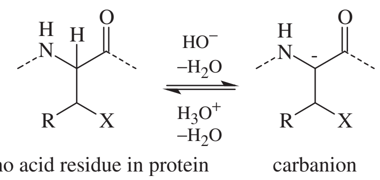


Figure 2.39 Racemisation of amino acids.

toxins, for extraction of nucleic acids, removal of residual meat from bones, production of protein isolates, peeling fruits and vegetables or during debittering of olives. In sterilised alkali-treated olives, significant amounts of D-Asx, D-Glx, D-Ser, D-His, D-Arg, D-Ala, D-Tyr, D-Val, D-Phe, D-Ile, and D-Leu were found. D-Lys was not detected. The most-abundant D-amino acids were D-Asx, D-Glx, D-Ser and D-Leu. The total content of D-amino acids in olives averaged 260 mg/kg in the edible portion, which is 4.2% of the total content of the amino acids (L- and D-enantiomers, 6170 mg/kg edible portion).

The racemisation of amino acids bound in proteins is accompanied by β-elimination of certain functional groups in side chains of amino acids, which yields **dehydroproteins** and subsequently cross-links in protein molecules (see Section 2.5.1.3.4). Racemisation begins with α-proton elimination of protein-bound amino acids, giving rise to an immediately isomerisable intermediate carbanion. This carbanion reacts with a proton (hydronium ion), which gives an equimolar mixture of the D-enantiomer and the original L-amino acid bound in protein. Proteolysis then releases the free D-amino acid (Figure 2.39).

The formation of D-amino acids reduces the nutritional value of the protein, because proteins containing D-amino acids have reduced digestibility by the organism and hence lower availability. The reason is the different methods of absorption, while L-amino acids are absorbed very efficiently in the digestive tract by active transport, D-amino acids are absorbed by passive diffusion, the effectiveness of which is limited. The toxicity of D-isomers is insignificant, with the exception of D-serine, which is reportedly nephrotoxic.

Racemisation of L-amino acids (elimination of an α-proton of protein bound amino acids) occurring *in vivo* during aging and some diseases (Alzeheimer's disease, cataracts and diabetes) is facilitated by reducing sugars. An example is the reaction of D-glucose shown in Figure 2.40. In essence, the Amadori product formed in the Maillard reaction (see Section 4.7.5) isomerises and is hydrolysed to a mixture of L-and D-amino acids under the formation of 3-deoxy-D-*erythro*-hexos-2-ulose or 1-deoxy-D-*erythro*-hexo-2,3-diulose.

2.5.1.3 Elimination and other reactions

2.5.1.3.1 Decarboxylation

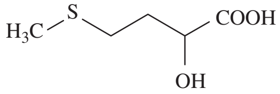
Amino acids eliminate carbon dioxide under the action of specific decarboxylases and, to a lesser extent, during heating to temperatures around their melting point (at around 200 °C). The products of decarboxylation of amino acids are the corresponding amines (Figure 2.41). Amines can also form by transamination of aldehydes catalysed by transaminases (see Section 8.2.10.1.2).

Aliphatic amines (e.g. methylamine formed from alanine and isobutylamine arising from valine) are very often flavour-active compounds of non-acidic foods, such as some cheeses. Amines derived from aromatic, heterocyclic and basic amino acids are biologically active compounds; therefore, they are collectively termed biogenic amines (see Section 10.3.2.8). An example of a biogenic amine is histamine, which is formed by decarboxylation of histidine. Histamine is involved, among other things, in immunological reactions of the organism. In meat and fish, biogenic amines are formed mostly as products of bacterial contamination. In foods obtained by fermentation processes (such as wine and sauerkraut), biogenic amines form by the activity of the microorganisms employed. A significant group of biogenic amines are the so-called polyamines, some of which (e.g. spermidine and spermine formed from putrescine or agmatine, the arginine decarboxylation product) play the roles of growth factors in both eukaryotic and prokaryotic cells. Phenylethylamine (the decarboxylation product of phenylalanine) is a precursor of catecholamines (dopamine, norepinephrine, or noradrenaline, and epinephrine, or adrenaline), a group of hormones secreted by the adrenal glands (see Section 10.3.2.8.1).

2.5.1.3.2 Transamination and oxidative deamination

Enzymatic reactions

Transamination catalysed by aminotransferases (formerly called transaminases) or oxidative deamination catalysed by oxidases transforms α-amino acids into the corresponding 2-oxoacids (Figure 2.42). Dehydrogenases transform 2-oxoacids into the corresponding secondary alcohols. For example, the product of glutamic acid oxidative deamination is 2-oxoglutaric acid, serine yields pyruvic acid and 2-oxobutanoic acid arises from threonine. Transamination of 2-oxobutanoic acid gives 2-aminobutyric acid. Certain bacteria, yeasts (including the yeast *Saccharomyces cerevisiae*) and fungi produce 2-hydroxy-4-(methylthio)butanoic acid (**2-115**) from methionine via reduction of the corresponding oxoacid. It is therefore a natural component of foods and feeds (in amounts of up to 60 mg/kg) produced by fermentation processes, such as fermented milk products, beer, bread and silage. In the form of its calcium salt, this methionine hydroxy analogue is used to fortify animal feeds. In the body, it is transformed into methionine.



2-115, 2-hydroxy-4-(methylthio)butanoic acid

Under the catalysis of ligases of the acid-thiol type, 2-oxoacids are transformed into acyl-CoA esters or by the action of decarboxylases (carboxylyases) into aldehydes having one carbon atom less than the original amino acid. For example, decarboxylation of pyruvic acid yields acetaldehyde and decarboxylation of 2-oxobutanoic acid gives propanal. The same aldehydes also form by Strecker

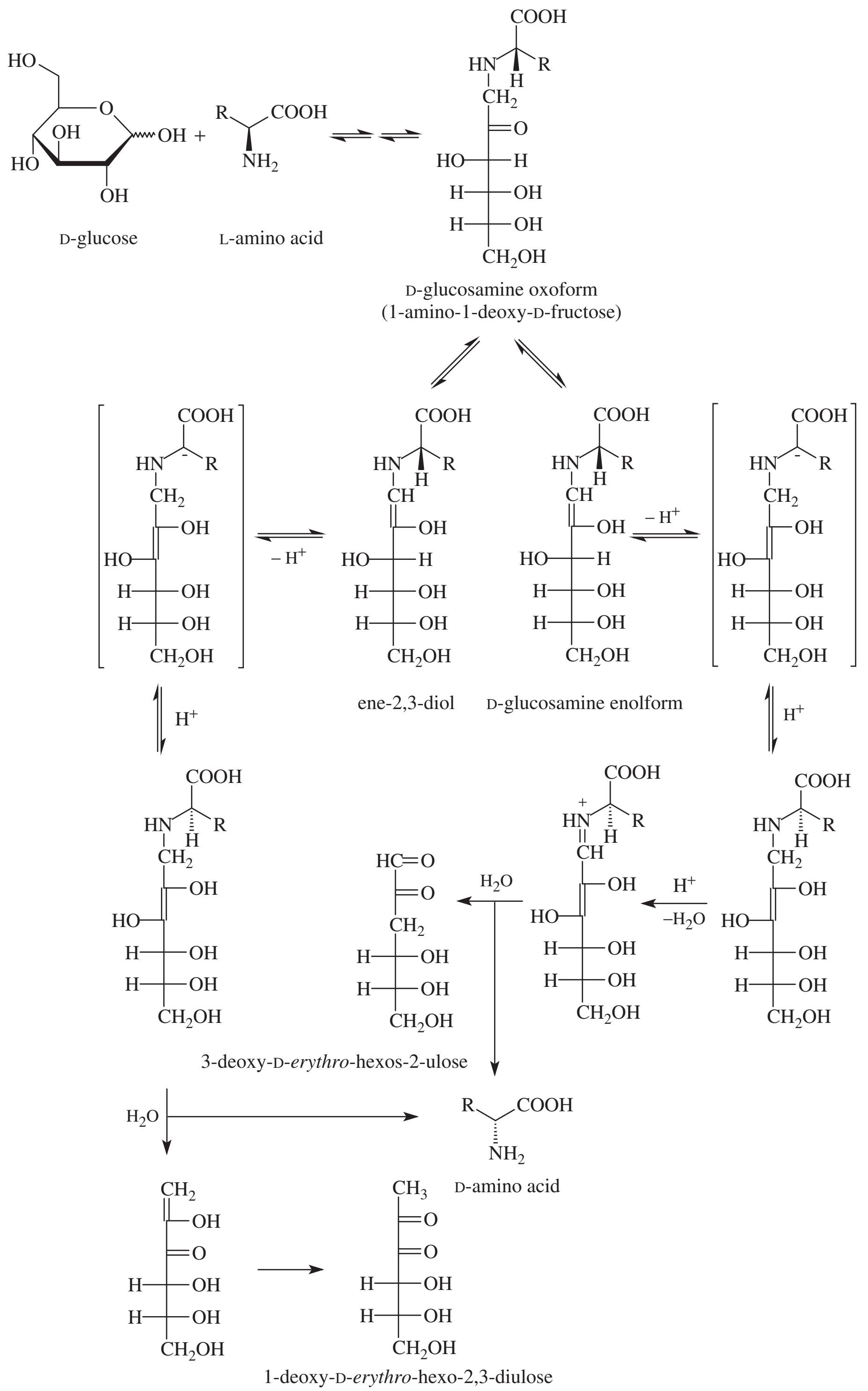


Figure 2.40 Isomerisation of L-amino acids catalysed by reducing sugars.

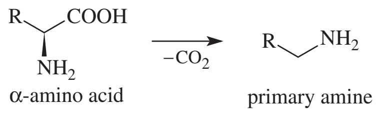


Figure 2.41 Decarboxylation of amino acids.

degradation of amino acids, which proceeds without catalysis by enzymes. Reduction of aldehydes by aldehyde dehydrogenases yields the corresponding primary alcohols. These can react with acyl-CoA esters (in the reaction catalysed by alcohol acyltransferases) to form carboxylic acids esters. Oxidation of aldehydes by aldehyde dehydrogenases provides carboxylic acid, and the

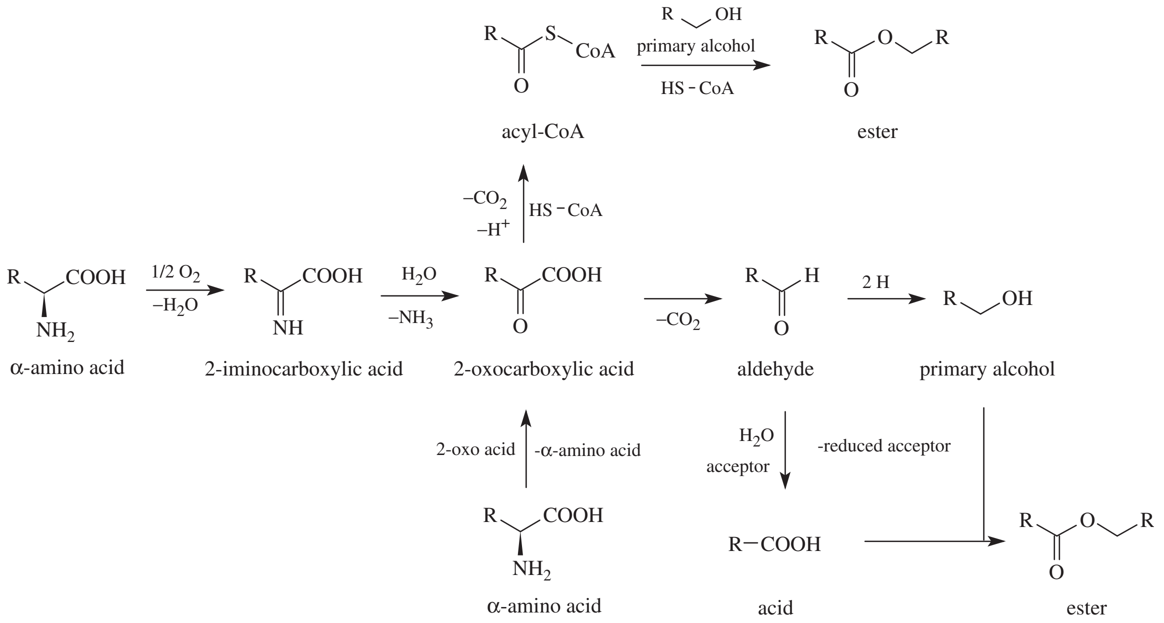


Figure 2.42 Formation of aldehydes, alcohols, carboxylic acids and their esters.

reaction with alcohols catalysed by carboxylesterases yields esters of carboxylic acids.

Aldehydes, alcohols, carboxylic acids, their esters and other transformation products of amino acid are important food volatiles. Aldehydes and fusel oil alcohols formed during alcoholic fermentation are important flavour-active compounds of alcoholic beverages. Esters of carboxylic acids are important flavour constituents of many foods, especially fruits, alcoholic beverages and dairy products. Aldehydes are also formed in the Maillard reaction during cooking, baking, frying and other thermal operations.

Strecker degradation

Oxidation of amino acids by oxidising agents, which generates carbon dioxide, ammonia and a carbonyl compound containing one less carbon atom than the starting amino acid, is called a **Strecker degradation** of amino acids (Table 2.26).

Monoaminomonocarboxylic α-amino acids with a primary amino group produce sensory active aldehydes called Strecker aldehydes. Strecker degradation of β-amino acids yields alkan-2-ones known as methylketones (see Section 8.2.4.1.2). By analogy, alkane-3-ones (ethylketones) are formed from γ-amino acids. The general reaction is schematically indicated in Figure 2.43. The reaction mechanism, however, varies considerably depending on the type of oxidant and amino acid. 2-Imino acids and 2-oxoacids can in some cases apparently form as intermediates, analogous to enzymatically catalysed transamination and oxidative deamination of amino acids (see Section 2.5.1.3.2). Some Strecker aldehydes readily decompose, such as methional, or yield cyclic products, such as 5-aminopentanal, which dehydrates to 2,3,4,5-tetrahydropyridine.

Inorganic oxidising agents, such as hypochlorites found in some sanitation and disinfection agents, may oxidise amino acids only in exceptional circumstances. Important oxidising agents are

Table 2.26 Strecker aldehydes produced from α-amino acids.

Amino acid	Degradation product	Amino acid	Degradation product
Glycine	Formaldehyde (methanal)	Cysteine	2-Mercaptoethanal
Alanine	Acetaldehyde (ethanal)	Methionine	3-Methylthiopropanal (methional)
Valine	2-Methylpropanal	Aspartic acid	3-Oxopropionic acid
Norvaline	Butanal	Glutamic acid	4-Oxobutyric acid
Leucine	3-Methylbutanal	Lysine	5-Aminopentanal
Norleucine	Pentanal	Ornithine	4-Aminobutanal
Isoleucine	2-Methylbutanal	Phenylalanine	2-Phenylethanal (phenylacetaldehyde)
Serine	Glycolaldehyde	Tyrosine	4-Hydroxyphenylacetaldehyde
Threonine	2-Hydroxypropanal	3,4-Dihydroxyphenylalanine	3,4-Dihydroxyphenylacetaldehyde

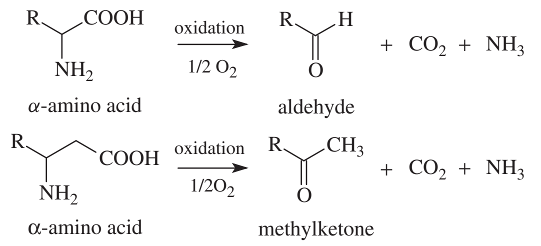


Figure 2.43 General scheme of Strecker degradation of amino acids.

mainly organic compounds, which contain electronegative functional groups in the molecule. The active substances in the Strecker degradation of amino acids in foods are therefore also fatty acid hydroperoxides, unsaturated aldehydes and ketones, derivatives of furan-2-carbaldehyde, α-hydroxyaldehydes and α-hydroxyketones and sugars (aldoses and ketoses). The active substance is also pyruvic acid, L-dehydroascorbic acid, quinones such as benzoquinones, naphthoquinones and anthraquinones and, therefore, also some compounds belonging to vitamins K or vitamins E (oxidised to tocopheryl quinones). The active substances are also some unsaturated lipid oxidation products, such as epoxyoxoene fatty acids.

Strecker degradation of amino acids is an extremely important reaction that occurs during storage and particularly during thermal processing of food. The main products of this reaction (Strecker aldehydes) are important volatiles in a variety of foods and many other aromatic and flavouring substances are formed by subsequent reactions of Strecker aldehydes and other Strecker degradation products, especially α-aminocarbonyl compounds, ammonia, amines and various sulfur compounds. Strecker degradation of amino acids also has its negative side, which is a loss of some essential amino acids (valine, leucine, isoleucine, threonine, methionine and phenylalanine).

2.5.1.3.3 *Elimination of ammonia, hydrogen sulfide and water*

α-Aminocarboxylic acids

When heated to higher temperatures (above their melting points) and in acidic solutions, α-amino acids condense through the formation of linear and cyclic dipeptides, cyclic six-membered amides known as 3,6-disubstituted 2,5-dioxopiperazines (see Section 2.3.3.2 and Figure 2.14).

β-Aminocarboxylic acids

Heating β-amino acids leads to the elimination of ammonia and to the formation of alk-2-enoic acids (α,β-unsaturated) acids (Figure 2.44). For example, aspartic acid behaves as a β-amino acid and produces (*E*)-but-2-enedioic acid, known under the trivial name fumaric acid. The reverse reaction, in which fumaric acid gives aspartic acid, occurs in living organisms and is catalysed by the enzyme aspartase. Analogously, elimination of ammonia from asparagine produces fumaric acid monoamide, known as

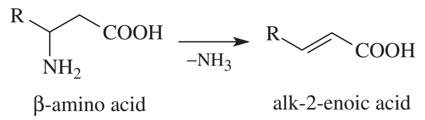


Figure 2.44 Formation of alk-2-enoic acids from β-amino acids.

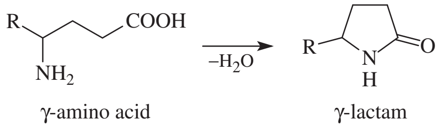


Figure 2.45 Formation of γ-lactams from γ-amino acids.

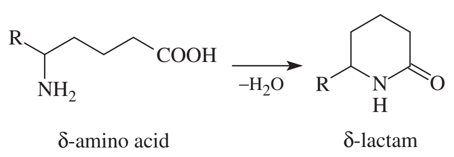


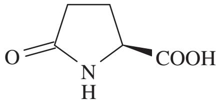
Figure 2.46 Formation of δ-lactams from δ-amino acids.

fumaramic acid. Its decarboxylation could theoretically produce toxic acrylamide. However, acrylamide is produced in reactions of asparagine with reducing sugars (known as the Maillard reaction) that enable its decarboxylation (see Section 12.2.2).

γ-Aminocarboxylic acids and δ-aminocarboxylic acids

The heating of γ- and δ-amino acids leads to intramolecular condensation and the formation of *N*-analogues of lactones, termed lactams. γ-Lactams (butane-4-lactams or pyrrolidine-2-ones) form from γ-amino acids (Figure 2.45), while δ-amino acids yield δ-lactams (pentane-5-lactams or piperidine-2-ones) (Figure 2.46).

Glutamic acid and glutamine behave as γ-amino acids, as they are easily transformed into L-5-oxopyrrolidin-2-carboxylic acid (also known as pyroglutamic acid, glutiminic acid or 5-oxoproline, 2-116) and its ammonium salt, respectively, during the thermal processing of foods. Pyroglutamic acid reportedly exhibits umami-like taste. The free acid occurs at levels of up to 300 mg/kg in fresh vegetables and fresh and preserved fruits, and in concentrations of 500–3000 mg/kg in canned vegetables, for example in pickled beets. The pyroglutamic acid content can serve as a qualitative index to estimate the proportion of tomatoes (refractometric dry matter introduced by the raw material) in tomato products, for example in the production of tomato paste (ketchup) by the hot-brake technology that includes preheating of tomatoes and straining of tomato seeds, as well as other operations conducted at elevated temperatures (such as thickening, puree



2-116, L-5-oxopyrrolidine-2-carboxylic acid

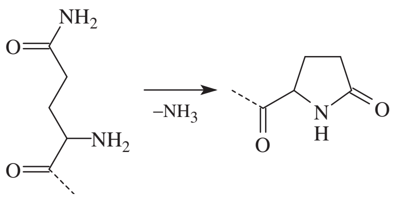


Figure 2.47 Transformation of *N*-terminal glutamine.

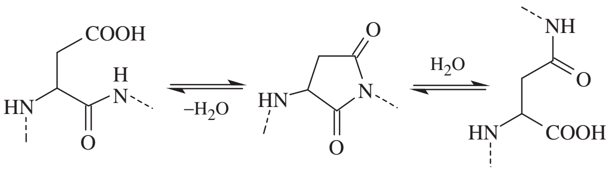
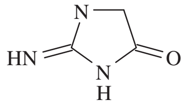


Figure 2.48 Interconversion of aspartic acid carboxylic groups.

pasteurisation, stirring and ketchup pasteurisation). Fresh tomatoes typically contain less than 10 mg/kg pyroglutamic acid. During processing, its content increases by one to two orders, and the final concentration of pyroglutamic acid in tomato juice is 25–40 mg/kg, in ketchup 150–220 mg/kg and in puree 630–820 mg/kg, depending on the tomatoes content.

Transformation (spontaneous cyclisation) of the *N*-terminal glutamine into *N*-terminal pyroglutamate also occurs in proteins (e.g. in κ-casein and *para*-κ-casein) during thermal processing, and takes place especially easily in acidic solutions (Figure 2.47). Aspartic acid only undergoes interconversion of α- and β-carboxylic groups under the same conditions (Figure 2.48).

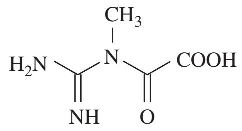
On heating the γ-amino acid creatine (**2-46**) yields an imidazolidine derivative known by the trivial name of creatinine (**2-117**). For example, fresh beef muscle contains creatine in amounts ranging from about 0.30 to 0.60% (according to the muscle type), while the creatinine content is only 0.020–0.040%. The creatine content of pork meat is lower than in beef, being 0.25–0.37%, while the creatinine content is only 0.003–0.009%. However, when the meat is cooked, the creatine content decreases whilst the creatinine content increases: the total content of these two substances might therefore be a useful indicator for quantifying the heat treatment applied in the processing of meat products. For example, cooked ham contains 0.29–0.32% of creatine and 0.032–0.073% of creatinine.



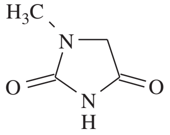
2-117, creatinine

The quality criteria for beef extract specify the minimum creatinine content as 8.5% (dry matter, added salt excluded) for use in the production of bouillon cubes. Creatine and creatinine levels may be therefore used as a measure during the detection of beef extracts in food products. Serum creatinine concentration is the most frequently used clinical estimate of renal function.

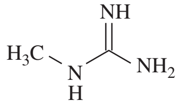
The product of creatine degradation via *N*-methylguanidinoglyoxylic acid (creatone, **2-118**) is oxalic acid and *N*-methylguanidine (**2-119**), which in fresh pork, beef, chicken and fish is found at levels of 1–10 mg/kg. *N*-Methylguanidine occurs in larger amounts (20–180 mg/kg) in some Japanese dishes prepared from smoked and dried fish. In the production of beef and whale meat extracts, which are used in soup seasoning preparations, creatinine produces small amounts of the amino acid sarcosine (*N*-methylglycine, **2-12**) and urea. Deamidation of creatinine by bacteria or in alkaline solutions can yield the toxic *N*-methylhydantoine (**2-120**). Creatinine is also a precursor of toxic aminoimidazoazaarenes found mainly in grilled meat and fish (see Section 12.2.1.1).



2-118, *N*-methylguanidinoglyoxylic acid



2-119, *N*-methylguanidine



2-120, *N*-methylhydantoine

Hydroxyamino acids

Under acidic conditions and especially during acid hydrolysis of proteins, serine and threonine isomerise to imino acids and β-elimination of water molecule yields the corresponding 2-oxoacids (Figure 2.49). Thus serine produces pyruvic acid (R = H) and threonine yields 2-oxobutanoic (2-oxobutyric) acid (R = CH_3). Decarboxylation of these oxocarboxylic acids at elevated temperatures yields acetaldehyde and propanal, respectively. Thermal degradation of serine, for example, has been reported to produce a wide range of products. The initial thermal degradation products include pyruvic acid, glycine and formaldehyde (formed by retro aldol condensation), 2-aminoethanol (formed by decarboxylation) and acetaldehyde (formed by elimination of ammonia from 2-aminoethanol). A number of products then arise in reactions of these initial products. Reaction of pyruvic

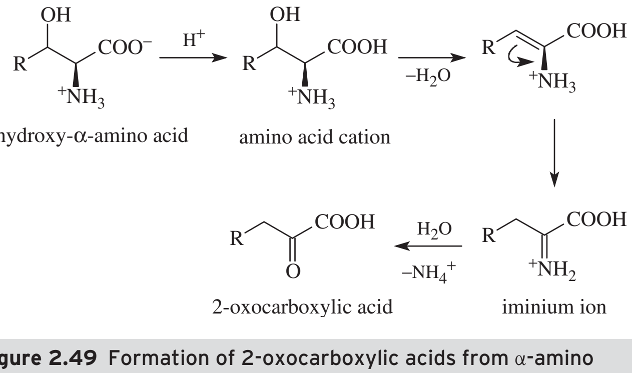


Figure 2.49 Formation of 2-oxocarboxylic acids from α-amino acids.

acid with 2-aminoethanol produces alanine and glycolaldehyde, reaction of acetaldehyde with 2-aminoethanol yields glycolaldehyde, ethylamine and 2-methyl-2-oxazoline, aldol condensation of formaldehyde to glycolaldehyde forms glyceraldehyde and reactive α-hydroxycarbonyl compounds are involved in the formation of heterocyclic compounds such as substituted pyrazines and pyrroles and some other heterocyclic compounds.

Amino acid amides

At temperatures of around 100 °C, asparagine and glutamine side chains react with the side chain of bound lysine (Figure 2.50) with the elimination of ammonia (the reaction is termed deamidation) and the peptide chains are connected by intermolecular and intramolecular transverse covalent bonds. These bonds, known as isopeptide bonds, consist of dipeptides ε-*N*-(β-aspartyl)-L-lysine and ε-*N*-(γ- glutamyl)-L-lysine, respectively. About 15% of lysyl residues can react in this way. The digestive proteases of some animals (e.g. chickens and rats) can split these bonds, but for humans the so-called bound lysine is not available. The result is the certain, but usually not very significant, reduction of the nutritional value of proteins. Reduction of the nutritional value can be important in cases where a diet is low in protein and the limiting amino acid of the protein is lysine. The Maillard reaction often leads to an extensive loss of lysine.

Sulfur amino acids

Free cystine is relatively stable at temperatures around 100 °C. The reaction that is observable during thermal processing of foods at temperatures of approximately 100 °C is partial elimination of hydrogen sulfide from bound cystine, which is often called protein desulfuration (Figure 2.51). This reaction splits the disulfide bridges in protein with the formation of cysteine and sulfenic acid residues. The unstable sulfenic acid intermediate is hydrolysed with the formation of hydrogen sulfide, yielding a reactive molecule containing a free carbonyl group. This compound reacts with the ε-amino group of lysine, which results in a clear loss of cystine (cysteine) and lysine. The collagen-bound lysine reacts similarly (see Section 2.4.5.1.3). At higher temperatures or in alkaline solutions, even under milder conditions, bound cysteine eliminates hydrogen sulfide yielding dehydroprotein as an intermediate (Figure 2.52). The resulting hydrogen sulfide is involved in the formation of many flavour-active compounds in thermally processed foods (see Section 8.2.9.1.1). Similarly to protein bound cysteine, some other protein bound amino acids can eliminate small molecules, yielding dehydroprotein. For example, protein bound methionine eliminates dimethyl sulfide.

If allowed by the structure of the protein bound amino acids, the heat treatment of protein or reaction in an alkaline solution produces carbanion as an intermediate in the pathway to D-amino acids (see Section 2.5.1.2) and dehydroprotein (Figure 2.52). The most frequently eliminated groups are the cysteine thiol group (X = SH), hydroxyl group of serine (X = OH) and phosphorylated hydroxyl group of phosphoserine (X = OPO_3H_2), which yield dehydroprotein containing bound 2-aminoacrylic acid, known as dehydroalanine (R = H). The reactive side chains of bound amino acids, such as side chains of lysine, ornithine and cysteine, can add to the double bond of the dehydroprotein, which leads to cross-linking of polypeptide chains in the protein (Figure 2.53).

Cross-linked proteins produce unusual amino acids by proteolysis. Bound lysine yields lysinoalanine (**2-121**), cysteine lanthionine (**2-122**) and ornithine ornithinoalanine (**2-123**). Bound ornithine forms in proteins (together with urea) by alkaline hydrolysis of arginine. In the same way, many other amino acids (arginine, histidine, threonine, serine, tyrosine and tryptophan) and also amines and ammonia react with dehydroprotein.

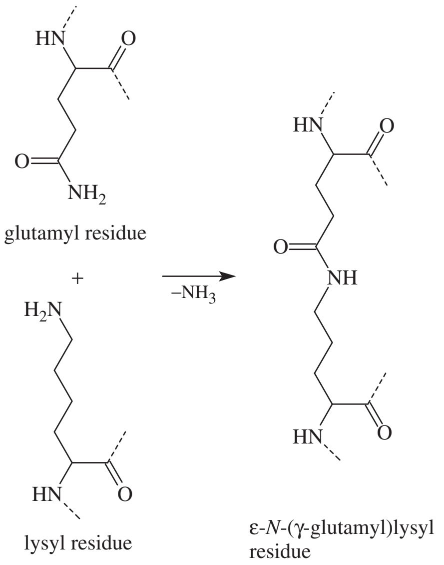


Figure 2.50 Deamidation of proteins.

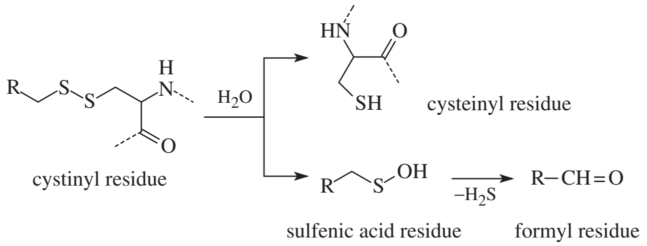


Figure 2.51 Desulfuration of proteins.

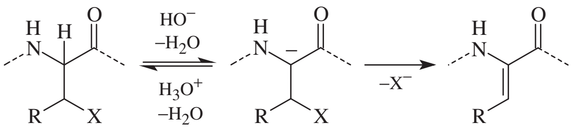


Figure 2.52 Formation of dehydroprotein (R = H or CH_3, X = OH, OR, SH, SR, S–SR, etc.).

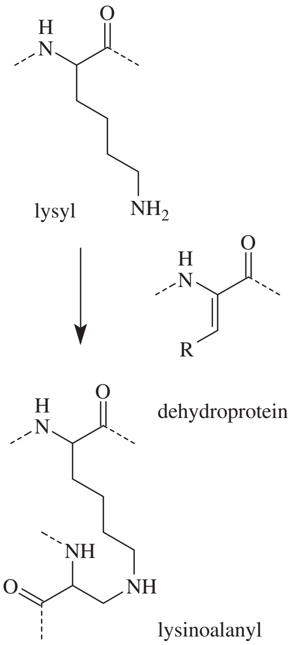
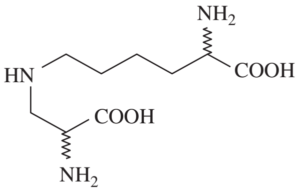
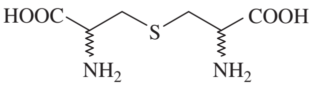


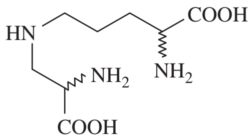
Figure 2.53 Formation of cross-links in proteins.



2-121, lysinoalanine



2-122, lanthionine



2-123, ornithinoalanine

These unusual amino acids are not bioavailable and therefore reduce the nutritional value of proteins. Some products are even toxic, especially lysinoalanine. In theory, the hydrolysis of protein containing bound L-lysine produces the same amount of free L,L- and L,D-lysinoalanine. Furthermore, the isomerisation of bound L-lysine produces bound D-lysine and protein hydrolysis then gives free D,L- and D,D-lysinoalanine. It is suggested that alkali treatment of protein might have important toxicological implications for man. Free lysinoalanine is partly excreted in the urine and partly catabolised in the kidney. Feeding lysinoalanine to rats induces changes in kidney cells characterised by enlargement of the cytoplasm and nucleus, designated as nephrocytomegaly (karyomegaly). The toxicity of the individual isomers differs. The least toxic is D,D-lysinoalanine, about three times more toxic is L,L-isomer, ten times more toxic is D,L-isomer and about 30 times more toxic is L,D-lysinoalanine. Lysinoalanine and related amino acids of protein cross-links are present in many foods, especially foods processed at high temperatures and in alkaline media (Table 2.27).

Table 2.27 Lysinoalanine content in some foods.

Food	Content (g/kg protein)	Food	Content (g/kg protein)
Soybean meal (defatted)	1.5	Sausage (boiler)	0.05
Soy protein (texturised)	0.3	Sausage (baked)	0.2
Soy protein (isolate)	0.4–11.6	Chicken (raw)	0.0
Milk evaporated	0.4–0.9	Chicken (baked)	0.1
Casein (acidic)	0.1–0.2	Egg white (original)	0.02
Sodium caseinate	0.3–6.9	Egg white (boiled)	0.1–0.4
Calcium caseinate	0.2–4.3	Egg white (baked)	0.4–1.1
Sausage (original)	0.0	Egg white (dried)	0.2–1.8

The peptide nisin, for example, contains *meso*-lanthionine (see Section 11.2.1.2.1).

More than 50 xenobiotic amino acids are known. Ammonia, which adds to dehydroprotein to form 2,3-diaminopropionic acid (β-aminoalanine, **2-41**), was used in the prevention of lysinoalanine and other cross-link amino acids formation.

2.5.2 Reactions with food components

2.5.2.1 Reactions with minerals

Polypeptide chains of proteins and peptides and free amino acids contain basic functional groups (typically amino or imino groups) and acidic functional groups (usually carboxyl groups or phosphoric acid residues in phosphoproteins), and therefore react with acids and bases. Salts of amino acids and proteins with acids and bases are stable only in a certain range of pH values. High molecular weight complexes can also be formed between basic and acidic proteins and between proteins and some polysaccharides. The most important reaction is the formation of salts and complexes with metal ions. Metal ions, however, often act as cofactors of enzymes and constitute a natural part of many complex proteins, such as milk caseins. Formation of salts and complexes of proteins with various metal cations (sodium, potassium, calcium) is employed in various food technologies, for example in production of caseinates and soybean curd (tofu). The formation of protein salts with various inorganic metal ions affects the solubility of proteins. Sodium, potassium and ammonium salts are predominantly soluble, while calcium salts are less soluble, and salts of heavy metals are mostly insoluble. Particularly strong complexes (their stability depends on pH and the conformation of polypeptide chains) are formed by cysteine, tyrosine and basic amino acids, lysine and histidine, therefore proteins, peptides and amino acids often act as natural antioxidants of lipids and other oxylabile substances because they react with ions of heavy metals, such as Fe^{3+} and Cu^{2+} that act as

active catalysts in the oxidation of lipids. A few simple examples of the structures of metal complexes are given in Section 6.2.2.1.

In some cases, however, the formation of salts and complexes is an unwanted phenomenon, as protein salts and complexes with polyphenols form hazes and sediments in preserved fruit, fruit juices and beer. Protein hazes can also form in white wines after bottling, where the main factor is inorganic sulfate ions. Protein complexes with transition metals are often coloured, and their formation in processed foods is generally undesirable. An example is the protein conalbumin occurring in egg white, which readily forms coloured complexes with metal ions (pink with Fe^{3+}, yellow with Cu^{2+} and Mn^{3+}) in media of pH $\geq$ 6 through tyrosine and histidine residues of the polypeptide chain. Complexes with iron ions often cause discoloration of egg products, but in media with pH < 4, these complexes dissociate to the original colourless compounds.

In addition to oxidation reactions (summarised in Section 2.5.1.1), the side chains of amino acids residues in proteins are readily oxidised through the catalysis of metal ions.

2.5.2.2 Reactions with oxidised lipids

Oxidised lipids react with each other and also with non-lipid food components under the conditions used in culinary and technological processing and even during storage and *in vivo*. Hydroperoxyl free radicals and epoxy and aldehyde functional groups of oxidised lipids are mainly involved in the reactions with proteins (also with peptides and free amino acids), reacting mainly with sulfhydryl, amino, guanidyl and imidazole functional groups of proteins and to a small extent with their peptide bonds. Reaction of proteins with oxidised lipids results in products of decreased solubility and digestibility, and often lead to unwanted discolourations, especially in fish.

Reaction products of reactive aldehydes derived from oxidised lipids, such as acrolein, (*E*)-4-hydroxynon-2-enal and malondialdehyde, with lysine, arginine and other amino acids are described as examples in Section 4.7.5.6. These products, ALE (advanced lipoxidation end products), formed *in vivo* are markers of oxidative stress in the organism. Reaction mechanisms are discussed in Section 3.8.1.12.1. As the final reaction products, proteins and oxidised lipids also form dark insoluble macromolecular products that contain variable proportions of lipid and protein fractions. In particular, such products include protein oligomers, proteins with oxidised sulfur amino acids, proteins containing imine bonds (C=N) formed by reaction with aldehydes or hydroperoxides (they mostly arise from the ε-amino group of bound lysine) and lipoproteins, in which lipids and proteins are bound by covalent bonds in contrast to native lipoproteins.

The amino groups of free amino acids and *N*-terminal amino groups of proteins also react with free fatty acids and triacylglycerols, yielding the corresponding fatty acid amides. These may be precursors of non-volatile nitroso compounds (nitrosamides) classified as food contaminants (see Section 12.2.7.1).

2.5.2.3 Reactions with aldehydes, ketones and sugars

2.5.2.3.1 Reactions with aldehydes

α-Amino groups of free amino acids, *N*-terminal α-amino groups of amino acids bound in peptides and proteins and ε-amino group of bound lysine react with carbonyl compounds (such as aldehydes, ketones and reducing sugars). The reaction starts with the addition of the amino group of the amino acid to the carbonyl group of the carbonyl compound to form imines, formerly known as Schiff bases (Figure 2.54). Subsequent reactions of the imines lead to a variety of reaction products. The reaction with reducing sugars, the Maillard reaction, is the most important reaction that takes place in foods during processing (see Section 4.7.5).

An important factor influencing the reactivity of amino acids and carbonyl compounds is pH. Reactivity of amino acids also depends greatly on basicity, or more precisely on the nucleophilicity, of the amino group, but also on other factors that are described in detail in Section 4.7.5.

Amino acids with reactive functional groups in the side chains, both aromatic and heterocyclic amino acids, react with aldehydes with the formation of a variety of heterocyclic products. Common food components that appear in foods during production are aldoses and various aliphatic, aromatic and heterocyclic aldehydes (such as formaldehyde, acetaldehyde, benzaldehyde and 2-furancarbaldehyde) and vanillin and anisaldehyde, which are added to foods as flavouring agents. With formaldehyde, cysteine gives thiazolidine-4-carboxylic acid, also known as thioproline. Higher aldehydes yield C-2 substituted (2*RS*,4*R*)-thiazolidine-4-carboxylic acids (Figure 2.55).

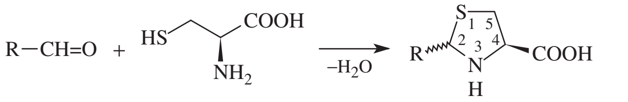


Figure 2.55 Reaction of cysteine with aldehydes.

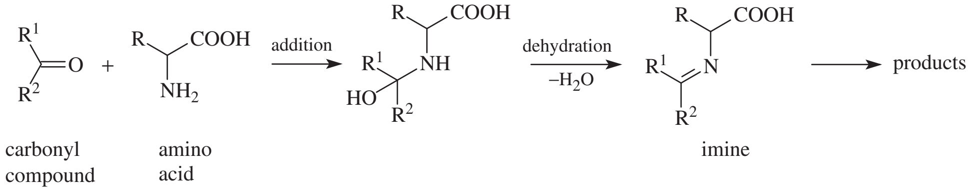
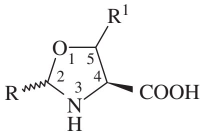
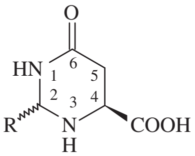


Figure 2.54 Reaction of amino acids with carbonyl compounds.

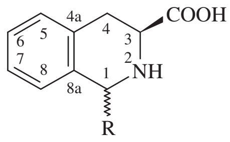
2-Methylthiazolidine-4-carboxylic acid, a condensation product of cysteine and acetaldehyde, occurs even in human blood as a consequence of ethanol consumption. Serine and threonine analogously produce C-2 substituted (2*RS*,4*S*)-oxazolidine-4-carboxylic acids (**2-124**). Heterocyclic products, C-2 substituted (2*RS*,4*S*)-pyrimidine-4-carboxylic acids, are also produced in the reaction of aldehydes with asparagine (**2-125**). Phenylalanine yields C-1 substituted (1*RS*,3*S*)-tetrahydroisoquinoline-3-carboxylic acids (**2-126**) and analogous products arise from tyrosine. Tryptophan reacts with aldehydes under the formation of 9*H*-pyrido[3,4-b]indole (also known as β-carboline or norharmane) derivatives, (1*RS*,3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids (**2-127**, R = H or alkyl or residues of other aldehydes and sugars), the reaction of tryptamine yields the corresponding (1*RS*)-1,2,3,4-tetrahydro-β-carbolines.



2-124, oxazolidine-4-carboxylic acids
R^1 = H, serine derived products
R^1 = CH_3, threonine derived products

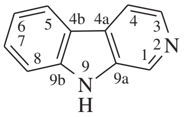


2-125, pyrimidine-4-carboxylic acids

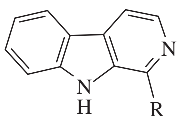


2-126, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acids

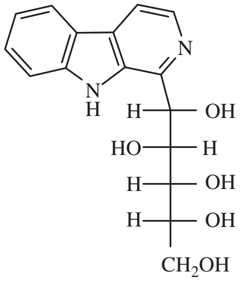
Of special importance are two types of these heterocyclic products, thiazolidine-4-carboxylic acids and β-carboline derivatives that may react with nitrogen oxides or nitrites with the formation of nitroso compounds, which can occur in foods as contaminants (see Section 12.2.7). Tryptophan-derived 1,2,3,4-tetrahydro-β-carboline and β-carboline alkaloids have been increasingly found in mammalian tissues and fluids, as well as in many foods of different origins, alcoholic and non-alcoholic drinks, fruit-derived products and tobacco smoke. Biochemical studies have revealed several actions of β-carbolines, including inhibition of monoamine oxidase, which catalyses the oxidative deamination of monoamines, competitive inhibition of monoamine neurotransmitter serotonine uptake, general inhibition of Na^+ dependent transports, binding to benzodiazepine (a psychoactive drug) and opiate receptors. They may act as neuromodulators and some may have an endocrinological function.



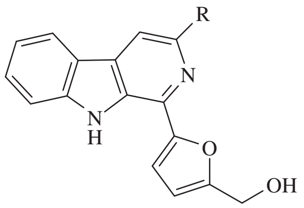
β-carboline



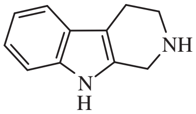
norharmane, R = H
harmane, R = CH_3



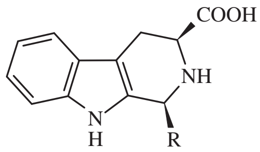
1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline



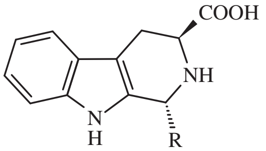
perloryline, R = H
flazine, R = COOH



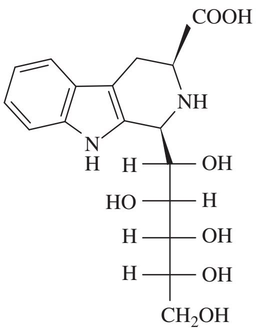
1,2,3,4-tetrahydro-β-carboline



(1*R*,3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids



(1*S*,3*S*)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids



(1*R*,3*S*)-1-(D-*gluco*-1,2,3,4,5- pentahydroxypentyl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid

2-127, β-carboline derivatives

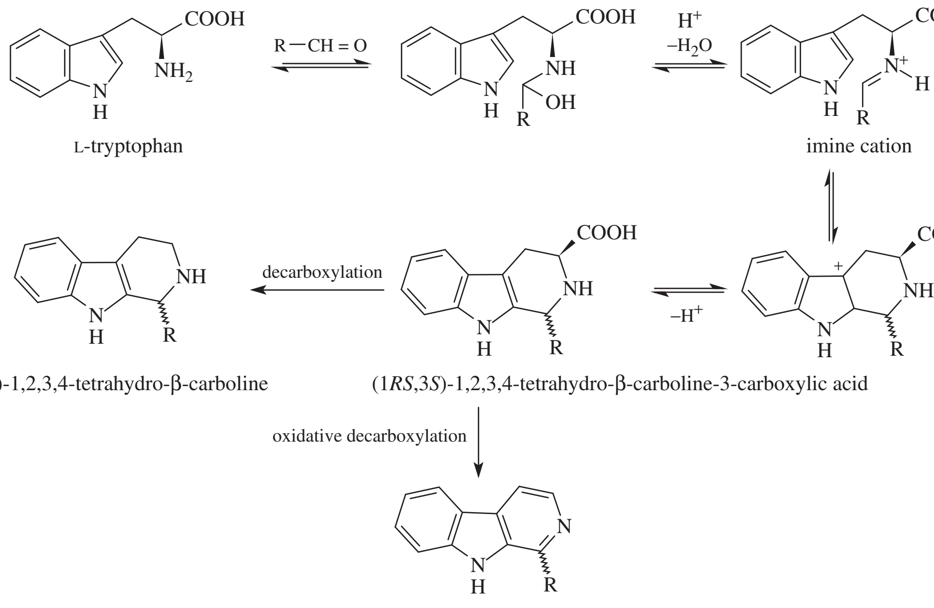


Figure 2.56 Reaction of tryptophan with aldehydes.

Tryptophan-derived 1,2,3,4-tetrahydro-β-carboline and β-carboline alkaloids result in foods through a Pictet–Spengler condensation (a special case of the Mannich reaction) of indoleamines, such as tryptophan and tryptamine, with aldehydes. The mechanism of the Pictet–Spengler reaction leading to 1,2,3,4-tetrahydro-β-carboline and β-carboline alkaloids is given in Figure 2.56.

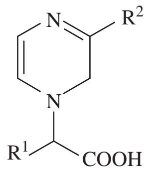
1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acids derived from formaldehyde and acetaldehyde and their decarboxylation products, 1,2,3,4-tetrahydro-β-carboline (**2-127**, R = H) and 1,2,3,4-tetrahydro-1-methyl-β-carboline (**2-127**, R = CH_3) have been identified in various products, such as cashew nuts, walnuts, pineapple, banana, wine, vinegar, beer, soy sauce, fruit juices, syrups, purees and jams. For example, 1,2,3,4-tetahydro-β-carboline-3-carboxylic acid and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, as two diastereoisomers (1*S*,3*S* and 1*R*,3*S*), have been found in commercially prepared fruit juices at levels of 0.01–1.45, 0.02–9.1 and 0.01–2.48 μg/g for 1,2,3,4-tetahydro-β-carboline-3-carboxylic acid, (1*S*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and (1*R*,3*S*)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, respectively. The content was higher in citrus juices (orange and grapefruit) than in other juices (grape, apple, pineapple, peach, banana, pear and tomato).

Decarboxylation and oxidation products of 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acids derived from formaldehyde, 9*H*-pyrido[3,4-*b*]indole (norharmane, **2-127**), and acetaldehyde, 1-methyl-9*H*-pyrido[3,4-*b*]indole (harmane, **2-127**), were identified in a number of foods at levels up to 700 mg/kg, although more typically their concentrations in smoked, cooked and fermented foods range from a few mg/kg to 1–2 orders of magnitude less. Typical norharmane and harmane findings in pan-fried, minced meat, beef patties or ground beef prepared at temperatures of 175–230 °C and cooking times 2–10 min ranged from 0.8 to 11.3 μg/kg and from 1.4 to 10.3 μg/kg, respectively. In raisins norharmane concentrations ranged from 2 to 120 μg/kg and concentrations of harmane were 6–644 μg/kg. Dark-brown raisins (sun-dried) contained higher levels of these alkaloids than golden raisins. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acids were identified in higher amounts, reaching up to 50 mg/kg. Arabica coffees contained norharmane and harmane at levels of about 136 and 51 μg/l, respectively. Commercial blends (usually with a maximum of 30% robusta) ranged from the cited arabica values to 345 μg/l of norharmane and to 145 μg/l of harmane. Higher levels were found in tobacco smoke, with 11.2 μg of norharmane and 3.6 μg of harmane per cigarette.

Reactions of tryptophan with aldohexoses (at temperatures above 50 °C and in acidic solutions) are a prerequisite for the formation of glycotetrahydro-β-carbolines that arise together with *N*-glycosides and *C*-glycosyl conjugates of tryptophan. Pictet–Spengler type condensation of tryptophan and glucose gives (1*R*,3*S*)- and (1*S*,3*S*)-diastereoisomers of 1-(D-*gluco*-1,2,3,4,5-pentahydroxypent-1-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (**2-127**). These compounds were also found in fruit juices. Grape juices or mixed fruit juices containing grape juice exhibited the highest level of pentahydroxypentyl-tetrahydro-β-carboline-3-carboxylic acids (up to 3.8 mg/l). Relatively high concentrations of these β-carbolines were also found in tomato, pineapple, multifruit and tropical fruit juices. In contrast, it was not detected in apple juices or banana and peach nectars, whereas a low level was detected in orange juices. In the presence of atmospheric oxygen or mild oxidants, such as L-dehydroascorbic acid, 1-(D-*gluco*-1,2,3,4,5-pentahydroxypent-1-yl)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid readily eliminates carbon dioxide and is oxidised to 1-(D-*gluco*-1,2,3,4,5-pentahydroxypent-1-yl)-β-carboline (**2-127**). Diastereoisomeric 1-(1,3,4,5-tetrahydroxypent-1-yl)-β-carbolines, 1-(1,4,5-trihydr-

oxypent-1-yl)-β-carbolines and *E*/*Z* isomers of 1-(1,5-dihydroxypent-3-en-1-yl)-β-carbolines, arising by oxidative decarboxylation and dehydration, isomerisation and reduction of the carbohydrate-derived side chain, were found in various food products. The highest concentrations of these β-carbolines were found in ketchups, soy and fish sauces. The main components were diastereoisomers of 1-(1,3,4,5-tetrahydroxypent-1-yl)-β-carbolines (1.4–3.5 mg/kg). β-Carbolines flazine and perlolyrine (**2-127**) derived from a degradation product of hexoses 5-hydroxymethylfuran-2-carbaldehyde were also found in soy sauce. Common concentrations of these alkaloids were 24 and 2.8 mg/kg, respectively.

Most of the reactions that occur in amino acids also take place in peptides and proteins. Heterocyclic products are formed when the *N*-terminal amino acid is involved in the ring formation. Products such as *N*-3 substituted 2-alkylpyrazin-2-ones form, for example, in reactions of peptides with glyoxal (**2-128**, R^1 and R^2 = peptide residues).



2-128, substituted pyrazin-2-one derivative

2.5.2.3.2 Reactions with sugars

The reaction with reducing sugars, the Maillard reaction, is the most important reaction that takes place in foods during processing. Reducing sugars are very reactive compounds. Their reactions with amino acids are described in detail in Section 4.7.5.1. For example, the reaction of amino acids with D-glucose yields imine as a primary product, which is transformed to the cyclic form D-glucosylamino acid and in a sequence of reactions splits off carbon dioxide, Strecker aldehyde and ammonia, while D-glucose is reduced to 2-deoxy-D-glucose. Another product of this reaction (in the absence of decarboxylation and cleavage of aldehydes) is the amino analogue of D-glucitol known as 1-amino-1-deoxy-D-glucitol (Figure 2.57). Strecker aldehydes can also be formed by oxidation of 1-amino-1-deoxyketoses (ketosamines) and by degradation of their oxidation products in the later stages of the Maillard reaction.

2.5.2.3.3 Reactions with dicarbonyl compounds

Particularly potent oxidants are α-dicarbonyl compounds, especially compounds produced by degradation of simple sugars and other food components (such as glyoxal and methylglyoxal) and their higher homologues, that is glycosuloses (aldoketoses and diketoses). The mechanism of oxidative decarboxylation of amino acids by α-dicarbonyl compounds is in fact more complex than that given in Figure 2.58. As in the reaction of other carbonyl compounds with amino acids, the first step is the addition of amino acid on the carbonyl group of an α-dicarbonyl compound with the formation of imine and elimination of water. The resulting imine then eliminates carbon dioxide and hydrolysis of the product yields Strecker aldehyde (preferably in a weakly acidic solution). Reduction of an α-dicarbonyl compound (transamination) produces a reactive aminocarbonyl compound. Other products include ammonia, amines, α-hydroxycarbonyl compounds, carboxylic acids and other compounds (Figure 2.58).

Carboxylic acids are formed in the presence of traces of transition metals, preferably in weakly alkaline solutions, and by oxidation with α-dicarbonyl fragments that are stronger oxidation agents than glycosuloses. The formation of carboxylic acids by autoxidation of

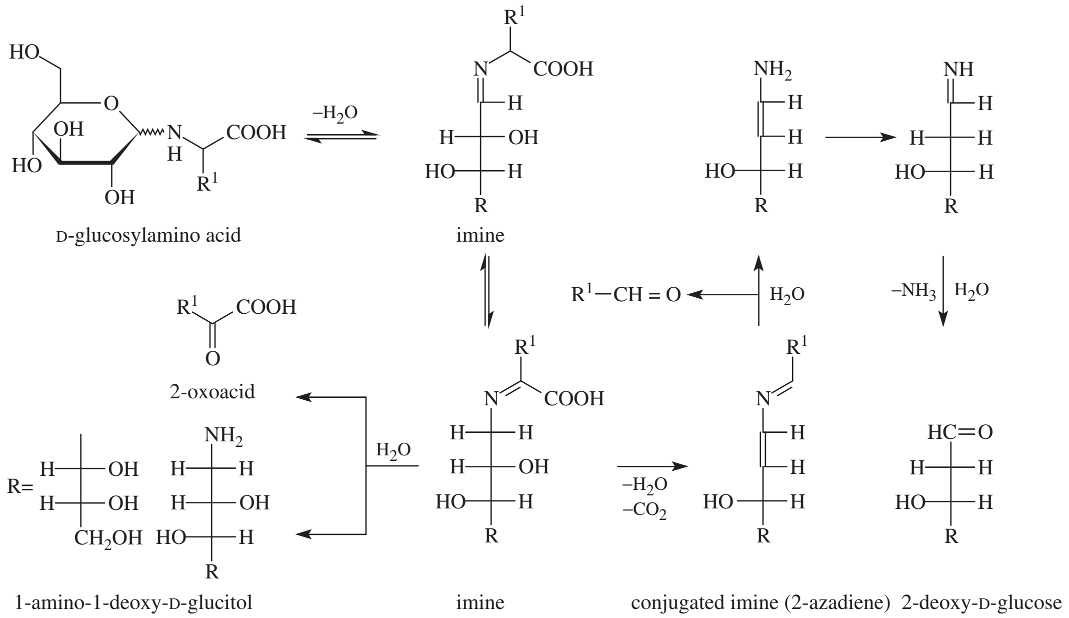


Figure 2.57 Strecker degradation of amino acids by aldoses.

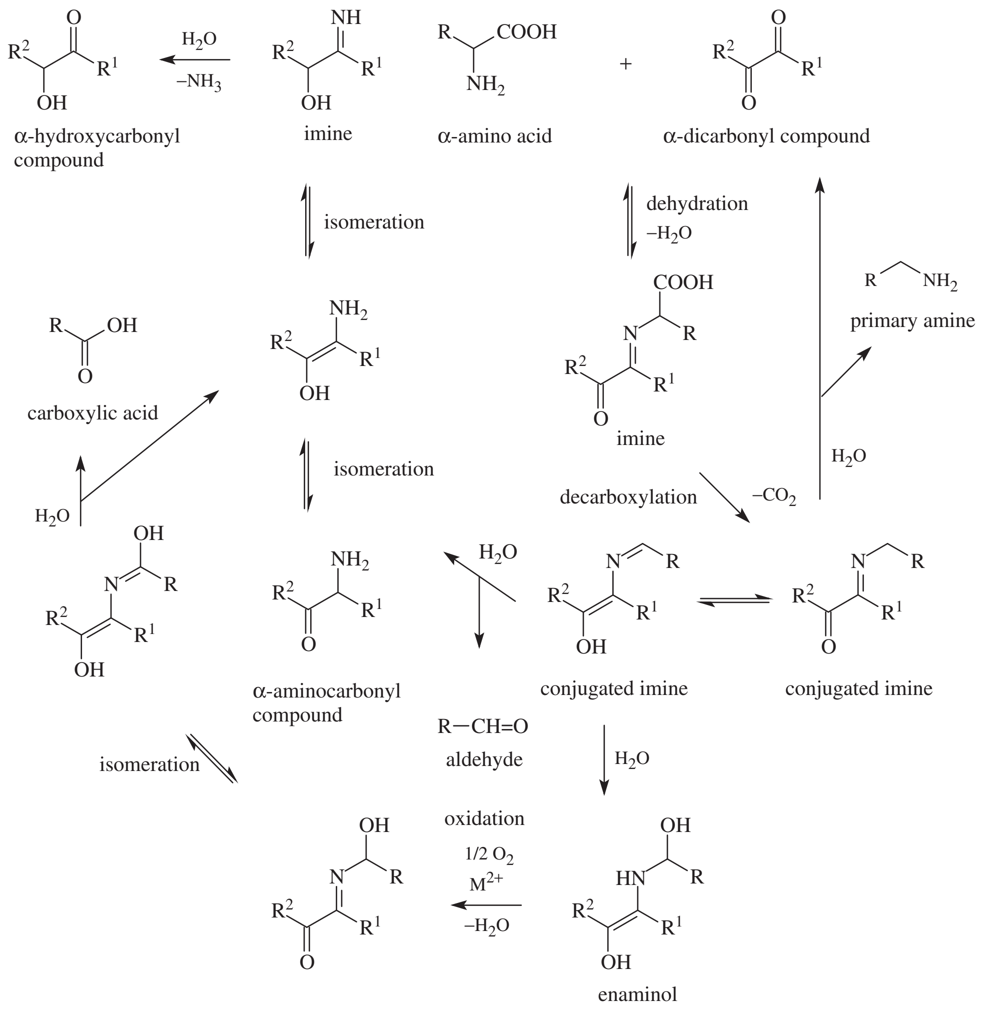


Figure 2.58 Strecker degradation of α-amino acids by α-dicarbonyl compounds.

Strecker aldehydes is not very significant, but some Strecker acids are important aroma components of cooked foods. The levels of amines formed by Strecker degradation, such as during roasting of cocoa beans, exceeds by several orders the amount of identical amines that are formed during fermentation and is comparable to the level of Strecker aldehydes generated.

The Strecker degradation of ornithine proceeds analogously, but the intermediate 4-aminobutanal forms by cyclisation the final product, 1-pyrroline (Figure 2.59). This reaction is important for the development of the characteristic aroma of bread crust (see Section 8.2.12.4.1), the aroma of other cereal products, certain fragrant rice varieties (such as Basmati rice) and in the biosynthesis of pyrrolidine alkaloids in plants (see Section 10.3.2.1.1). It was proposed that pyrrolidine and 1-pyrroline also result upon Strecker-type reaction of proline by α-dicarbonyl compounds.

In some cases, Strecker aldehydes are unstable and decompose to other products. For example, aspartic acid produces 3-oxopropionic acid (**2-129**), which provides acetaldehyde by decarboxylation. Other products are pyruvic acid and fumaric acid. Glutamic acid produces 4-oxobutyric acid (4-oxobutanoic acid, **2-130**), which eliminates carbon dioxide and yields propionaldehyde and other products, such as 2-oxobutyric acid and 5-oxopyrrolidine-2-carboxylic acid (**2-116**).

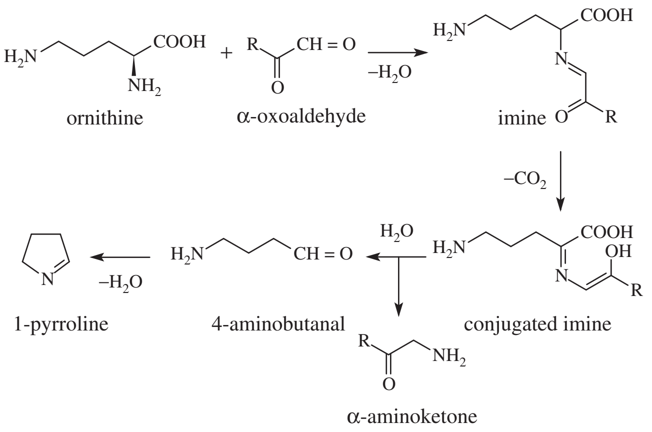


Figure 2.59 Strecker degradation of ornithine by α-dicarbonyl compounds.

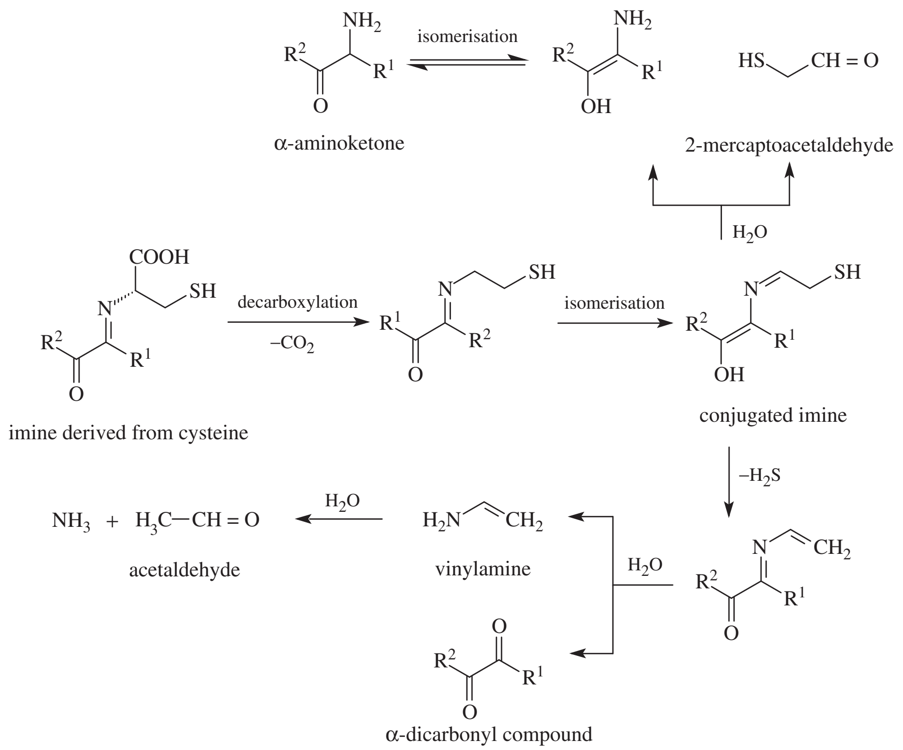
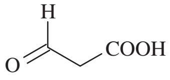
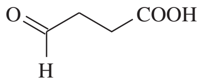


Figure 2.60 Strecker degradation of cysteine.

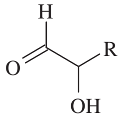


2-129, 3-oxopropionic acid



2-130, 4-oxobutanoic acid

More complicated is the Strecker degradation of hydroxyamino acids (serine and threonine) and of sulfur amino acids (cysteine and methionine). The Strecker degradation product of serine is glycolaldehyde (**2-131**), while threonine produces 2-hydroxypropanal (lactic acid aldehyde, **2-131**). Both aldehydes are highly reactive compounds that enter into a number of other reactions. Degradation of the imine of cysteine yields 2-mercaptoacetaldehyde and also vinylamine as a minor degradation product (Figure 2.60). Methionine (the corresponding imine) yields an unstable volatile aldehyde methional (3-methylthiopropanal), which is a characteristic odorous product of boiled potatoes. Methional further decomposes to acrolein and methanethiol (methylmercaptan), which spontaneously oxidises to dimethyldisulfide in the presence of oxygen (Figure 2.61). Oxidation of methional yields the corresponding sulfoxide, which decomposes via methanesulfenic acid to acrolein. Methanesulfenic acid reacts with methanethiol or other thiols producing dimethyldisulfide or mixed disulfides, respectively.



2-131, glycolaldehyde (R = H)
2-hydroxypropanal (R = CH_3)

Oxidising agents often cause degradation of amino acids and of the reaction intermediates by homolytic cleavage (homolytic fission) of their covalent bonds under the formation of free radicals, whose recombination creates a number of other compounds. For example, the main reaction product of the Strecker degradation of phenylalanine is phenylacetaldehyde, but also its lower homologue benzaldehyde and the higher homologue 3-phenylpropanal arise as minor products. Some other minor Strecker degradation products of phenylalanine include bibenzyl and *trans*-stilbene.

2.5.2.4 Reaction with isothiocyanates

Isothiocyanates are formed by the degradation of glucosinolates present in cruciferous vegetables (see Section 10.3.2.4.2). Free and bound peptides and proteins react with isothiocyanates via amino groups, mercapto groups or other nucleophilic functional groups (hydroxyl groups). Their reactivity depends, as well as the addition on the carbonyl group, on their nucleophility, pK_a and pH values.

Reactions of α-amino groups of amino acids with isothiocyanates in a weakly alkaline medium produce *N*,*N*′-disubstituted thioureas (*N*-thiocarbamoyl derivatives of amino acids) as primary products, which cyclise under acidic conditions, or on heating, to 2-thiohydantoines (Figure 2.62) that are considered to be responsible for some of the biological effects of isothiocyanates. In neutral and alkaline media, 2-thiohydantoines yield symmetric dehydrodimers as a result of cleavage of the acidic hydrogen atom in position C-5. 2-Thiohydantoines with the methylene group at C-5 ($R^1 = H$, Figure 2.63), derived from glycine, yield yellow to

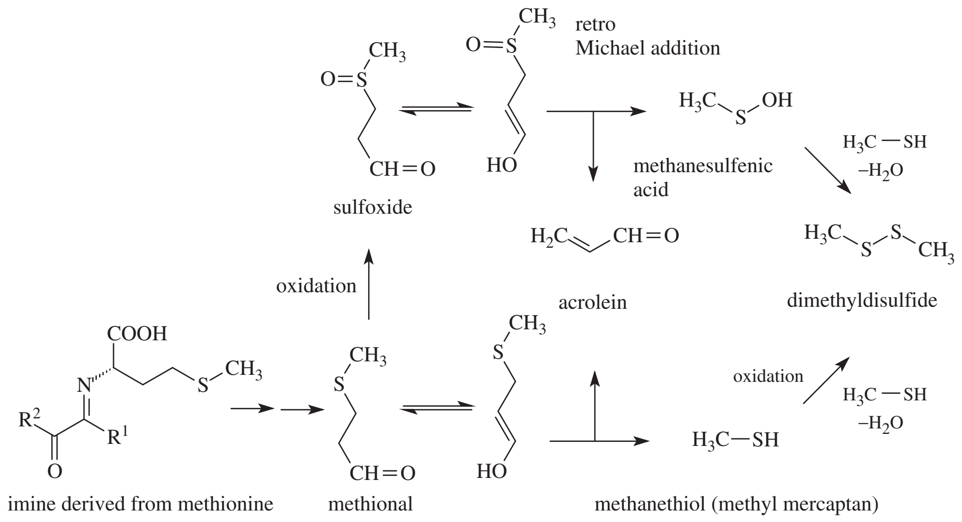


Figure 2.61 Strecker degradation of methionine.

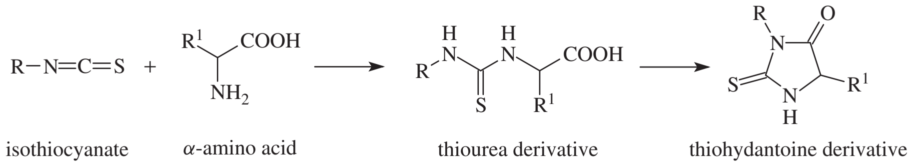


Figure 2.62 Reaction of α-aminokyselin with isothiocyanates.

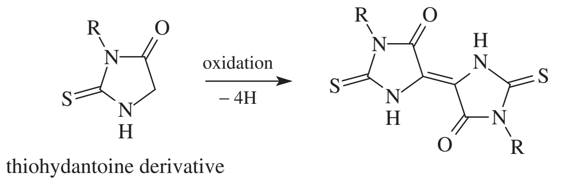


Figure 2.63 Formation of coloured 2-thiohydantoine dehydrodimers.

red diastereoisomeric 3,3′-dialk(en)yl- or 3,3′-diaryl-2,2′-dithioxo-[5,5′]-bisimidazolidinylidene-4, 4′-diones. Sarcosine actually yields blue products in alkaline solutions.

The reaction of isothiocyanates with cysteine gives (depending on pH) two types of primary products. In weakly acidic solutions (pH 5–6), isothiocyanate reacts exclusively with the thiol group of cysteine, producing *N*-substituted esters of dithiocarbamic acids and heterocyclic products. The same products are formed from cystine as from cysteine. Detoxification of isothiocyanates in the body is based on their reaction with reduced glutathione (G-SH) mercapto group. The resulting mercapturate is excreted in the urine.

In a weakly alkaline solution, isothiocyanate reacts with the α-amino group of cysteine (Figure 2.64). The reaction of isothiocyanates with cystine leads to the cleavage of disulfide bond (–S–S–) with the formation of cysteine sulfenic acid and a dithiocarbamic acid ester. Dehydration of this ester yields 5-amino-1,3-thiazine-4-one-3-thione derivative and elimination of hydrogen sulfide produces a cyclic 2-thiazoline-4-carboxylic acid derivative (Figure 2.65). Reaction of cystine with thiocyanates (R–S–C≡N), formed by spontaneous isomerisation of isothiocyanates, produces disulfide Cys–S–S–R and β-thiocyanoalanine, which cyclises to 2-thiazoline derivative (Figure 2.66).

Isothiocyanates also react with the hydroxyl group of tyrosine and other hydroxyamino acids. The addition of a hydroxy compound usually yields *N*-substituted thiocarbamates, which are esters of *N*-substituted thiocarbamoic acids (Figure 2.67).

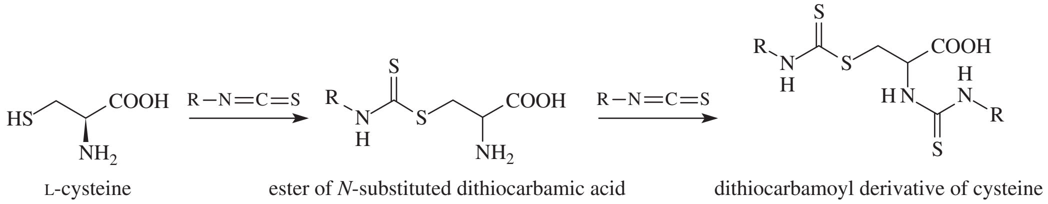


Figure 2.64 Reaction of isothiocyanates with cysteine.

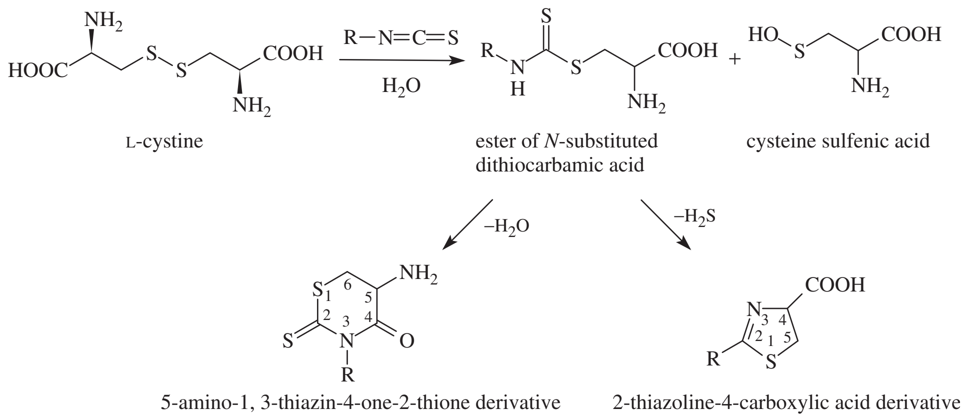


Figure 2.65 Reaction of isothiocyanates with cystine.

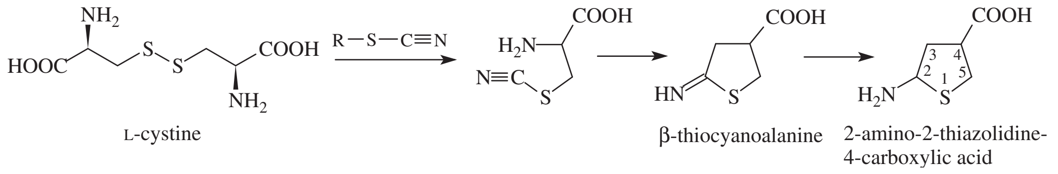


Figure 2.66 Reaction of thiocyanates with cystine.

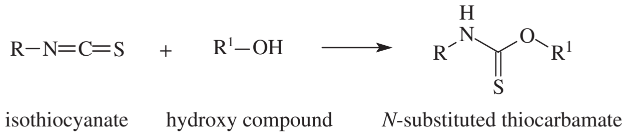


Figure 2.67 Reaction of isothiocyanates with hydroxy compounds.

Isothiocyanates react with *N*-terminal amino groups of peptides[13] to form thiourea, which spontaneously cyclises to imidazolidine derivatives under acidic conditions and the reaction product is the original peptide without *N*-terminal amino acids (Figure 2.68). Reactions of isothiocyanates with the *N*-terminal amino groups of proteins and other nucleophilic centres containing thiol, disulfide, ε-amino and hydroxyl groups can cause changes in protein conformations. These proteins are poorly hydrolysable in the protein conformation, which is related to the antimicrobial and antithyroid activity of isothiocyanates.

[13]Reaction with phenyl isothiocyanate (Edman degradation) has been of great significance in determining the primary structure of peptides.

2.5.2.5 Reaction with plant phenols

Phenolic compounds known as polyphenols (such as phenolic acids and flavonoids) are easily oxidised by atmospheric oxygen in reactions catalysed by oxidoreductases (*o*-diphenol:O_2 oxidoreductases). These reactions are known as enzymatic browning reactions (see Section 9.12). Autoxidation of plant phenols catalysed by heavy metal ions (mainly cupric and ferric ions) and oxidation by lipid hydroperoxides lead to similar products. The products of oxidation of the so-called *o*-diphenols (1,2-dihydroxybenzenes) are *o*-quinones (1,2-benzoquinones), highly reactive compounds that react with proteins (amino acids) and other food components with the formation of dark-coloured polymeric products resistant to proteolysis.

The reaction of *o*-quinone with amino or thiol groups of proteins (*N*-terminal group or ε-amino group of lysine) leads to adducts that are transformed into *o*-diphenols. They are very oxylabile and readily oxidise to the disubstituted *o*-quinones (Figure 2.69), which can react with other quinone or protein molecules to form oligomeric products. The reaction with thiol groups of proteins occurs in weakly acidic and neutral solutions. The reactions with amino groups of proteins in neutral solutions also lead to disubstituted *o*-quinones. In alkaline solutions, amino group adds to the

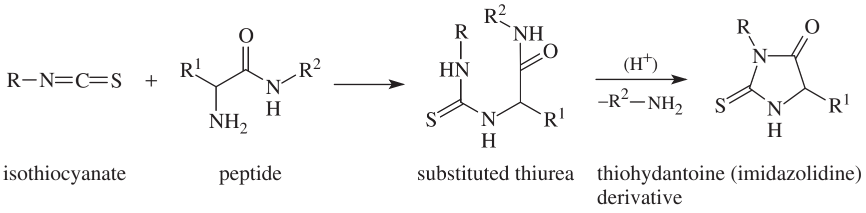


Figure 2.68 Reaction of isothiocyanates with peptides.

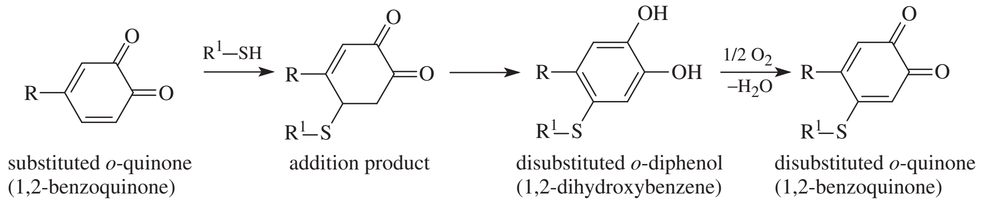


Figure 2.69 Reaction of 1,2-benzoquinones with protein thiol group.

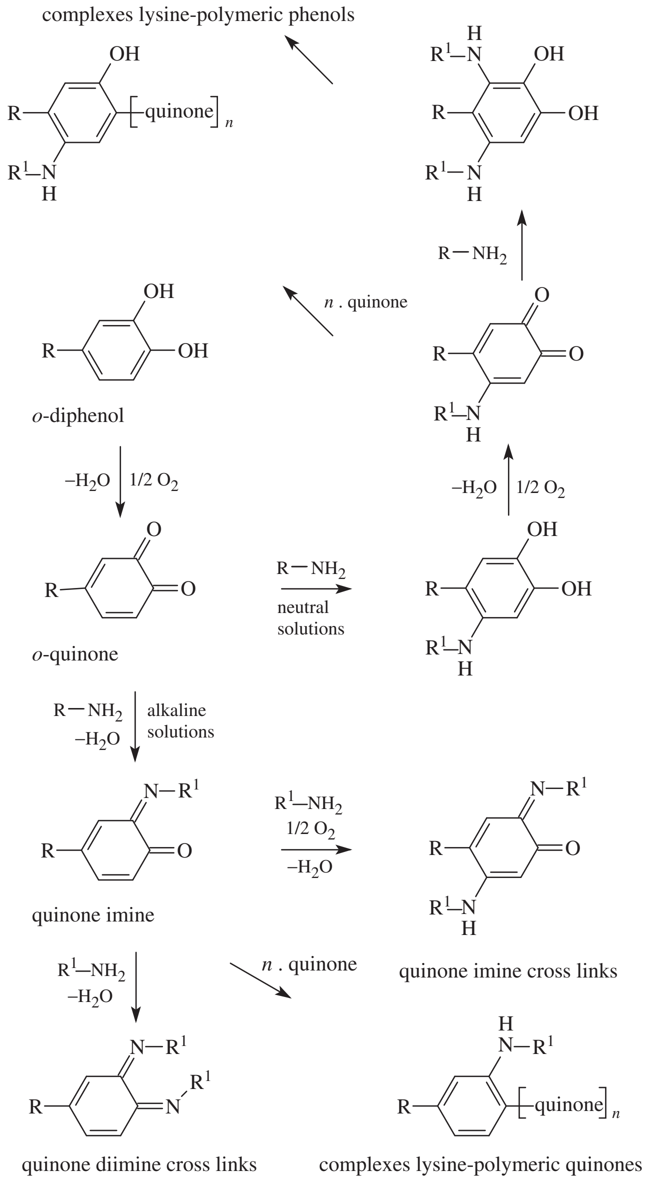


Figure 2.70 Reaction of lysine with o-quinones (R^1-NH_2 = ε-amino group of lysine). Stephen 1995, fig 10. Reproduced by permission of Taylor & Francis – Marcel Dekker.

carbonyl group of quinones, which results in replacement of one or both quinone oxygen atoms (=O) by an =N–R group and formation of quinonimines (quinone imines) (Figure 2.70). Strecker degradation of amino acids also takes place as a side reaction. The reaction of proteins with plant phenols during the extractions of oil meals (e.g. in sunflower meal, which contains a considerable amount of polyphenols) when extracting proteins from alkaline solutions and in the production of protein isolates, therefore, leads to a range of discolourations of the products and also reduces the protein nutritional value due to the loss of essential amino acids lysine (bound in the non-available products), methionine (by oxidation) and a lower availability of tryptophan, which is due to lower protein digestibility.

2.5.2.6 Hydrolysis and protein hydrolysates

Proteolytic enzymes are usually present in all raw food materials. Spontaneous hydrolysis of proteins therefore occurs during storage in a number of foods (e.g. in meat during ripening). Proteases also play a significant role in various plant materials. For example, they act during dough maturation, which affects the functional properties of gluten, in the production of malt and in other processes. Autolysis of fish meat, oysters and other marine animals, for example, is employed for the production of special Oriental sauces. Autolysates of waste brewery yeast are used as food additives. Various food technologies also employ proteolytic preparations from various microorganisms, for example chymosin is used in the production of some cheeses (see Section 2.4.5.2.3). Partial microbial protein hydrolysis is used for the production of soy sauces. Mineral acids are utilised to achieve almost complete hydrolysis of proteins to amino acids in the production of hydrolysed vegetable proteins for use as seasonings.

Soy sauce, or soya sauce, is the best-known representative of the **enzymatic protein hydrolysates**. It is made from soybeans (soya beans), roasted wheat, water and salt. The process of manufacture for naturally fermented soy sauce can be divided into four stages: preparation of koji, brine fermentation, filtration/pasteurisation and maturation. Koji is produced by cooking soya beans (defatted soya bean meal) and then mixing the resultant material with milled roasted wheat. The mixture is inoculated with a mould, either *Aspergillus oryzae* or *Aspergillus sojae*, cultured for 2–3 days at 25–30 °C, and then mixed with saltwater brine and yeast to give moromi. The moromi is fermented (2 days) and the resulting material is pressed to obtain the raw soy sauce, which is pasteurised, filtered and stored for a period of months in order to mature. Finally, the product is filtered, bottled and distributed.

Chemical protein hydrolysates, also called hydrolysed plant proteins or hydrolysed vegetable proteins, are typically produced by hydrochloric acid (HCl) hydrolysis of various proteinaceous vegetable raw materials (defatted oilseed meals, wheat gluten,

maize and rice protein and occasionally some animal proteins such as casein and keratin). The hydrolysis is usually carried out with 20% (6 M) HCl at temperatures exceeding 100 °C for up to about 8 hours. After cooling, the hydrolysate is neutralised by sodium hydroxide or sodium carbonate, generally to a pH ranging from 4.5 to 7. A product of this neutralisation is sodium chloride; its content in the hydrolysate is about 20%. The raw hydrolysate is filtered, it can be bleached and fortified with flavour-active compounds, and is then ripened for several weeks. The ripe product is then available as a liquid seasoning, alternatively it can be concentrated to a paste or dried to powder.

During production of the hydrolysate, amino acids react with sugars and other components of the original material, forming brown pigments called melanoidins or humins and a range of flavour-active compounds. Acid hydrolysis, however, leads to complete degradation of oxylabile tryptophan and glutamine and asparagine are hydrolysed to aspartic acid and glutamic acid, respectively. Approximately 5–10% of the hydroxyamino acids serine and threonine are degraded with the formation of 2-oxoacids and the cysteine is desulfurated to a large extent.

2.5.2.7 Other reactions

Food processing at higher temperatures during baking, frying, grilling and roasting leads to degradation of amino acids and production of many different products that significantly affect the smell, taste and colour of the product. Reactions are usually complex. There are several types of reactions taking place concurrently, and reaction products enter the mutual reactions and react with other food components.

Pyrolysates of amino acids and various foods processed at high temperatures (around 200 °C and above) contain some mutagenic and carcinogenic products, such as the technological contaminants acrylamide (the decomposition product of asparagine; see Section 12.2.2) and furan (formed from alanine, serine and other precursors; see Section 12.2.3) and aminoimidazoazaarenes (see Section 12.2.1.1), that are a result of creatinine and some Maillard reaction products. Other aromatic amines are generated by pyrolysis of amino acids such as tryptophan, glutamic acid, lysine, ornithine and phenylalanine. Structurally related heterocyclic products (derivatives of imidazopyrazines) were also found in the pyrolysates of aliphatic amino acids. Pyrolysis of amino acids and proteins at very high temperatures in burnt foods, but also by the burning of tobacco (around 800 °C) and other plant materials, generates a number of different products such as polycyclic aromatic hydrocarbons.

Amino acids also react with some food additives (such as sulfur dioxide), which results in the loss of cysteine and formation of non-available products. Toxic (carcinogenic) nitroso compounds are formed during the reaction of nitrites, nitrous acid or nitrogen oxides with amino acids containing a secondary amino group (*N*-alkylamino acids or imino acids). Toxic products are also formed in the reaction of methionine with some oxidising agents (nitrogen trichloride NCl_3) formerly used as food additives and in the reaction of cysteine with chlorinated solvent residues.

3
Fats, Oils and Other Lipids

3.1 Introduction

Lipids are one of the major components in foods and their role in human nutrition is one of the most important areas of concern in the field of nutritional science. Interestingly, however, lipids are such a diverse range of compounds that no single agreed definition exists; the main criterion for inclusion in this group of compounds is their hydrophobicity and not their chemical properties.

Lipids are usually defined as natural compounds containing bound fatty acids that contain more than three carbon atoms in the molecule. This definition is used by most biologically oriented workers, mainly physicians. However, ethyl esters of fatty acids with 4–12 carbon atoms, which are commonly found as odorous food components, are not classified as lipids. Some difficulty is caused by the inclusion of free fatty acids because they do not contain a bound alcohol residue. However, they are generally regarded as lipids and form a separate group together with their salts, which are known as soaps. Foods also frequently contain derivatives of fatty acids arising from industrial or other human activities (such as sugar esters and esters of sugar alcohols with higher fatty acids) that are not natural substances, but are still usually classified as lipids. Today, lipids are often defined as fatty acids and their derivatives, which are derived biochemically and related by their solubility in nonpolar solvents and general insolubility in water.

In practice, the non-volatile lipophilic compounds that accompany true lipids in both natural and manufactured products are also classified as lipids. They are termed **compounds accompanying lipids** (formerly called lipoids) in food chemistry, and 'unsaponifiable lipophilic substances' in food analysis. Their chemical structure is different and often these compounds do not even contain bound fatty acids. This group includes a large number of lipophilic compounds, for example some **terpenoids**,[1] especially steroids (see Section 3.7.4) and carotenoids (see Section 9.9). Compounds accompanying lipids also include lipophilic vitamins (see Chapter 5), some pigments (see Chapter 9), natural antioxidants (see Section 11.2.2) and other lipophilic compounds.

The following sections describe the individual classes of lipids and lipid accompanying substances, their structure, occurrence, isolation from natural sources and importance in nutrition and metabolism. Technological procedures used for production of lipid derivatives on an industrial scale are included. A large part of the chapter is devoted to the interactions of lipids with other food components and reactions that positively or negatively affect the nutritional value and organoleptic properties of foods. Attention is also paid to reactions of lipids under normal physiological conditions, such as during aging of living organisms and reactions linked with pathogenesis of certain diseases.

[1]Monoterpenoids, sesquiterpenoids and diterpenoids, despite their structural similarity with higher terpenoids, are not classified as lipids, although they are lipophilic. These compounds are described in detail in relation to food flavour (see Chapter 6).

3.2 Classification

According to their chemical composition, lipids are classified into three major groups[2]:

- **homolipids**
- **heterolipids**
- **complex lipids**.

[2]Recent classification systems define lipids as hydrophobic or amphipathic compounds biosynthesised solely or partially by condensation of thioesters (fatty acids and polyketides) or isoprenoid units (prenols, sterols and other compounds). Lipids are divided into two large groups, simple and complex lipids. Simple lipids provide at least two types of hydrolysis products (such as fatty acids and sterols or fatty acids and acylglycerols). The complex lipids (such as glycerophospholipids and glyceroglycolipids) provide three or more types of products through hydrolysis. According to the structure, lipids are divided into eight basic categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, steroid lipids, prenoid lipids, saccharolipids and polyketides.

The Chemistry of Food, First Edition. Jan Velíšek.
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Homolipids are compounds that contain fatty acids and alcohols. They are divided further according to the structure of the bound alcohol. Heterolipids are lipids that also contain, in addition to the fatty acids and alcohols, other covalently bound compounds, such as phosphoric acid in phospholipids or D-galactose in some glycolipids. Complex lipids contain homolipids and heterolipids, and, along with covalent bonds, some components are bound by various physical bonds, such as hydrogen bonds or hydrophobic interactions.

This classification is fairly logical in basic chemical or biochemical research of lipids, but in practice, however, this system is not often used. More frequently we come across a totally illogical designation such as 'neutral lipids' and 'polar lipids'. The neutral lipids include esters with glycerol, steroids and their esters, but also free fatty acids, although they are not neutral. Polar lipids include phospholipids, as well as many other heterolipids that often do not contain phosphorus or even fatty acids. This classification system of lipids is usually seen in biologically-oriented areas, and is based mainly on the behaviour of compounds during chromatographic separation. In food technology and oleochemistry, the name 'lipids' is not commonly used. Instead, only fats, oils, fatty acids, waxes and lecithin are recognised, since only these components have any industrial relevance.

3.3 Fatty acids

Fatty acids are the building blocks of lipids and nutritionally the most important lipid components. According to the terminology used in organic chemistry, the term fatty acids means carboxylic acids with an aliphatic hydrocarbon chain, but this definition does not correspond completely with the fatty acids present in lipids. Some fatty acids, according to the definition used by organic chemists (e.g. acetic acid), are not present in natural lipids, although they can occur in manufactured fat products. Fatty acids bound in lipids can, however, be alicyclic or even aromatic compounds.

3.3.1 Structure and nomenclature

In nature, and therefore also in foods, lipids contain the following groups of fatty acids:

- saturated fatty acids
- unsaturated fatty acids with one double bond (monoenoic)
- unsaturated fatty acids with several double bonds (polyenoic)
- fatty acids with triple bonds and with various substituents (alkynoic, branched chain and cyclic acids, acids with oxygen-, nitrogen- or sulfur-containing functional groups).

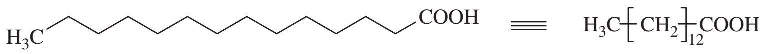
3.3.1.1 Saturated fatty acids

Saturated fatty acids are common constituents of natural lipids. They normally contain from four to 38 carbon atoms, but there are also higher fatty acids with about 60 carbon atoms, which are mostly linear (with unbranched chains) and usually have an even number of carbon atoms. In most natural lipids, saturated fatty acids constitute between 10 and 40% of the total fatty acids. Depending on the number of carbon atoms (chain length), saturated (as well as unsaturated) fatty acids can be divided into short chain fatty acids (C_4 and C_6), medium chain fatty acids (C_8–C_{12}), long chain fatty acids (C_{14}–C_{18}), very long chain fatty acids (C_{20}–$C2_6$) and ultra-long chain fatty acids (C_{28}–C_{38}).

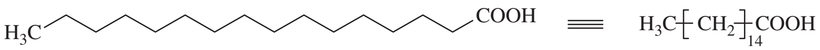
An overview of important fatty acids with an even number of carbon atoms in the molecule is given in Table 3.1. Besides the systematic names derived from the corresponding hydrocarbons, trivial names are also given, and these are used predominately in routine practice, especially in the nomenclature of common fatty acids. In the literature, for brevity, various short designations predominate, such as an N:M ratio, where N is the number of carbon atoms in the molecule and M the number of double bonds. The most abundant saturated fatty acids in animal and plant tissues

Table 3.1 Saturated fatty acids occurring in lipids.

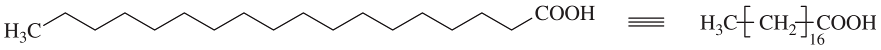
Fatty acid	Number of carbon atoms	Trivial name	Fatty acid	Number of carbon atoms	Trivial name
Butanoic	4	Butyric	Eicosanoic	20	Arachidic
Hexanoic	6	Caproic	Docosanoic	22	Behenic
Octanoic	8	Caprylic	Tetracosanoic	24	Lignoceric (carnaubic)
Decanoic	10	Capric (caprinic)	Hexacosanoic	26	Cerotic
Dodecanoic	12	Lauric	Octacosanoic	28	Montanic
Tetradecanoic	14	Myristic	Triacontanoic	30	Melissic
Hexadecanoic	16	Palmitic	Dotriacontanoic	32	Lacceric
Octadecanoic	18	Stearic	Tetratriacontanoic	34	Gheddic (geddic)



3-1, myristic acid



3-2, palmitic acid



3-3, stearic acid

are myristic (tetradecanoic, 14:0; **3-1**) acid, palmitic (hexadecanoic, 16:0; **3-2**) acid and stearic (octadecanoic, 18:0; **3-3**) acid.

Fatty acids with an even number of carbon atoms are accompanied by a small amount of fatty acids with an odd number of carbon atoms (most often they are fatty acids 13:0 to 19:0). The particularly common acids are pentadecanoic (15:0) and heptadecanoic (margaric, 17:0) acids, with pentanoic (valeric, 5:0), heptanoic (enanthic, 7:0), nonanoic (pelargonic, 9:0) acids and many others being present at low levels. These acids are found as esters in microbial lipids and in trace amounts in most animal tissues, at a concentration of 5% or more in tissues of ruminants.

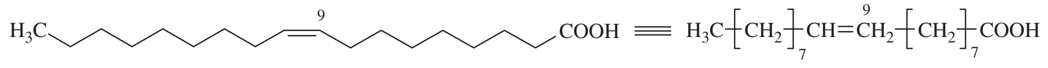
3.3.1.2 Unsaturated fatty acids with one double bond

Unsaturated fatty acids with one double bond, with the trivial name of monoenoic fatty acids, differ from each other by the number of carbon atoms, the double bond position in the hydrocarbon chain and the spatial organisation of molecules (*cis*–*trans* isomerism also known as *E*/*Z* isomerism or geometric isomerism). The most important of these are listed in Table 3.2. Many monoenoic fatty acids have trivial names, which are commonly used. A tpical example is *cis*- or (*Z*)-octadec-9-enoic acid, also known as oleic acid (**3-4**).

Many less common monoenoic fatty acids are only found in large amounts in inconsequential sources of lipids, but in foods they are usually present only in traces. For example, *cis*-hexadec-6-enoic acid (known as sapienic acid) is the most abundant fatty acid in human sebum, as among hair-bearing animals it is restricted to humans. Systematic names are often used for monoenoic fatty acids, especially when it is necessary to describe precisely the location and spatial configuration of the double bond in the molecule. For example, the major *cis*-octadec-9-enoic (oleic) acid is accompanied by a small amount of *cis*-octadec-11-enoic acid known as *cis*-vaccenic acid or asclepic acid, and *cis*-octadec-12-enoic acid appears to exist in hardened oils as a result of hydrogenation of linoleic acid so that only the double at the C-9 position is hydrogenated, whereas the double bond in the C-12 position remains unchanged. The short designation, 18:1, for all of these acids says nothing about the position of the double bonds. If we wish to indicate the position of the double bond, for example in oleic acid, the short designation

Table 3.2 Major monoenoic fatty acids accurring in lipids.

Fatty acid	Number of carbon atoms	Double bond position	Isomer	Trivial name
Decenoic	10	4	*cis*	Obtusilic
Decenoic	10	9	*cis*	Caproleic
Dodecenoic	12	4	*cis*	Linderic
Dodecenoic	12	9	*cis*	Lauroleic
Tetradecenoic	14	4	*cis*	Tsuzuic
Tetradecenoic	14	5	*cis*	Physeteric
Tetradecenoic	14	9	*cis*	Myristoleic
Hexadecenoic	16	6	*cis*	Sapienic
Hexadecenoic	16	9	*cis*	Palmitoleic
Hexadecenoic	16	9	*trans*	Palmitelaidic
Octadecenoic	18	6	*cis*	Petroselinic
Octadecenoic	18	6	*trans*	Petroselaidic
Octadecenoic	18	9	*cis*	Oleic
Octadecenoic	18	9	*trans*	Elaidic
Octadecenoic	18	11	*cis*	Asclepic (*cis*-vaccenic)
Octadecenoic	18	11	*trans*	*Trans*-vaccenic
Eicosenoic	20	9	*cis*	Gadoleic
Eicosenoic	20	11	*cis*	Gondoic
Docosenoic	22	11	*cis*	Cetoleic
Docosenoic	22	11	*trans*	Cetelaidic
Docosenoic	22	13	*cis*	Erucic
Docosenoic	22	13	*trans*	Brassidic
Tetracosenoic	24	15	*cis*	Nervonic (selacholeic)
Hexacosenoic	26	17	*cis*	Ximenic
Triacontenoic	30	21	*cis*	Lumequeic

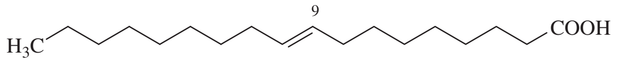


3-4, oleic acid

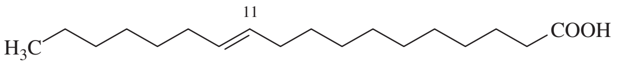
9-18:1 or 18:1Δ9 can be used, where the number 9 indicates that the double bond is at the ninth carbon atom from the carboxyl. The spatial configuration of natural fatty acids is usually *cis* (*Z*) or more rarely *trans* (*E*). Systematic names now increasingly use the newer designations of *Z* and *E*, but far more common (and still correct) is the designation *cis* and *trans*. Oleic acid is then *cis*-octadec-9-enoic acid or (*Z*)-octadec-9-enoic acid, also abbreviated as *c*-octadec-9-enoic acid, 9*c*-18:1 or 18:1 *cis*-9.

If the trivial name is used instead of the systematic name, it should be noted that this generally refers only to an isomer with a specific location of the double bond and steric configuration. Trivial names should therefore not be used when the individual isomers are not known, for example in the data on fat composition. In other words, the group of octadecenoic acids cannot be generally referred to as oleic acid, and it is not possible to speak of about 11-oleic acid, which does not exist. The designation isooleic acids has been applied to all isomers of octadecenoic acids, which have the double bond of the eighteenth carbon chain at some other place in the chain, instead of at C-9.

The prominent *trans*-unsaturated fatty acids are mainly (*E*)-octadec-9-enoic (elaidic) acid (**3-5**) and (*E*)-octadec-11-enoic (*trans*-vaccenic) acid (**3-6**).



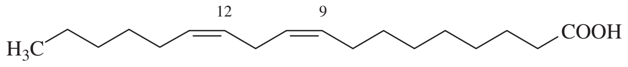
3-5, elaidic acid



3-6, *trans*-vaccenic acid

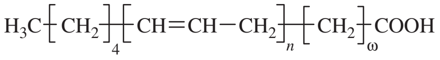
3.3.1.3 Unsaturated fatty acids with two or more double bonds

Fatty acids with two isolated double bonds separated by one methylene group (methylene interrupted double bonds) are very important in nutrition. Theoretically, there should be far more of these acids than of monoenoic fatty acids in natural lipids, but only a few are found in significant amounts (Table 3.3). The most important dienoic acid is linoleic acid (**3-7**). Again, positional as well as geometric isomers of polyenoic fatty acids exist. For example, linoleic acid is a 9*cis*, 12*cis*-isomer, that is, a 9-*cis*, 12-*cis*-isomer or (9*Z*,12*Z*)-octadeca-9,12-dienoic acid, abbreviated 9*c*12*c*-18:2 or 18:2 *cis*-9, *cis*-12.



3-7, linoleic acid

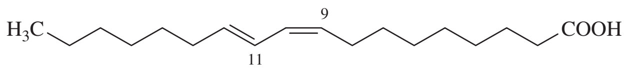
In addition, categorising polyunsaturated fatty acids according to the position of the double bond away from the terminal methyl group is common in biological documents. The nomenclature *n*-*x* (or *ω*-*x*) means that the first double bond is located on carbon number *x* counting from the terminal methyl group. The omega notation is common in popular nutritional literature. Linoleic acid is an example of a polyunsaturated fatty acid of the *n*-6 series (**3-8**). A short notation for linoleic acid is 18:2*n*-6 (or 18:2ω-6). Similarly, we come across fatty acids of *n*-3 (or ω-3) series (**3-9**).



3-8, polyenoic fatty acids of *n*-6 series (ω=2–6, *n*=2–5)

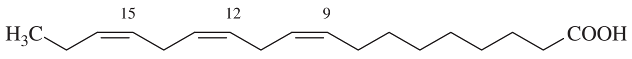
3-9, polyenoic fatty acids of *n*-3 series (ω=2–6, *n*=2–6)

Of particular importance are fatty acids with conjugated double bonds, which differ significantly from fatty acids with isolated double bonds in their reactivity and have distinct physiological effects (see Section 3.3.3.2). An example is (9*Z*,11*E*)-octadeca-9,11-dienoic acid (formerly known as conjugated linoleic acid or bovinic acid; the recommended trivial name is rumenic acid, **3-10**).



3-10, (9*Z*,11*E*)-octadeca-9,11-dienoic acid

The number of naturally occurring fatty acids with three *cis*-double bonds is considerably smaller than would be expected from the possibilities of isomerism (Table 3.3). The most important representative of these fatty acids is linolenic acid with three isolated double bonds (**3-11**). For example, linolenic acid is the 9*cis*,12*cis*,15*cis*-isomer, that is, (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoic acid, abbreviated to 9*c*12*c*15*c*-18:3 or 18:3 *cis*-9, *cis*-12, *cis*-15. In biological texts, it is often referred to as α-linolenic acid, which is a member of the *n*-3 series, unlike the isomeric *γ*-linolenic acid (octadeca-6,9,12-trienoic acid), a member of the *n*-6 series (**3-12**), which has different physiological effects. An example of conjugated fatty acids with three double bonds are (8*E*,10*E*,12*Z*)-9,11,13-octadecatrienoic (α-calendic) acid



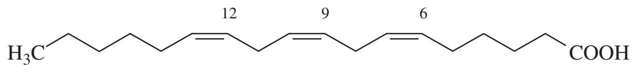
3-11, α-linolenic acid

Table 3.3 Dienoic, trienoic and other polyenoic fatty acids occurring in lipids.

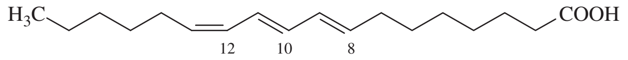
Fatty acid	Number of carbon atoms	Positions of double bonds	Isomer	Trivial name
Dienoic				
Hexadecadienoic	16	9,12	*cis*, *cis*	–
Octadecadienoic	18	9,12	*cis*, *cis*	Linoleic
Octadecadienoic	18	9,12	*trans*, *trans*	Linolelaidic
Octadecadienoic	18	12,15	*cis*, *cis*	–
Eicosadienoic	20	11,14	*cis*, *cis*	–
Dokosadienoic	22	13,16	*cis*, *cis*	–
Trienoic				
Hexadecatrienoic	16	6,10,14	all-*cis*	Hiragonic
Octadecatrienoic	18	6,9,12	all-*cis*	γ-Linolenic
Octadecatrienoic	18	8,10,12	*trans*, *trans*, *cis*	α-Calendic
Octadecatrienoic	18	8,10,12	*trans*, *trans*, *trans*	β-Calendic
Octadecatrienoic	18	9,11,13	*cis*, *trans*, *trans*	α-Eleostearic
Octadecatrienoic	18	9,11,13	*trans*, *trans*, *trans*	β-Eleostearic
Octadecatrienoic	18	9,11,13	*cis*, *trans*, *cis*	Punicic (trichosanic)
Octadecatrienoic	18	9,12,15	all-*cis*	α-Linolenic
Eicosatrienoic	20	8,11,14	all-*cis*	Dihomo-γ-linolenic
Tetraenoic				
Octadecatetraenoic	18	6,9,12,15	all-*cis*	Stearidonic (moroctic)
Octadecatetraenoic	18	9,11,13,15	*cis*, *trans*, *trans*, *cis*	α-Parinaric
Octadecatetraenoic	18	9,11,13,15	all-*trans*	β-Parinaric
Eicosatetraenoic	20	5,8,11,14	all-*cis*	Arachidonic
Docosatetraenoic	22	7,10,13,16	all-*cis*	Adrenic
Pentaenoic				
Eicosapentaenoic	20	4,8,12,15,19	all-*cis*	Clupanodonic
Eicosapentaenoic	20	5,8,11,14,17	all-*cis*	Timnodonic (EPA)
Docosapentaenoic	22	4,7,10,13,16	all-*cis*	–
Docosapentaenoic	22	7,10,13,16,19	all-*cis*	Clupadonic (DPA)
Hexaenoic				
Docosahexaenoic	22	4,7,10,13,16,19	all-*cis*	Cervonic (DHA)
Tetracosahexaenoic	24	6,9,12,15,18,21	all-*cis*	Nisinic

(**3-13**) and (9*Z*,11*E*,13*Z*)-octadeca-9,11,13-trienoic (punicic or trichosanic) acid (**3-14**).

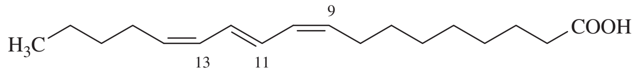
Highly unsaturated fatty acids with four to six *cis*-double bonds in the molecule (Table 3.3 and Figure 3.1) occur relatively rarely in nature. They belong to the *n*-3 and *n*-6 series. The most important of these fatty acids are those with four or five double bonds. The acid with four all-*cis* double bonds (*n*-6 series) is arachidonic (5,8,11,14-eicosatetraenoic) acid (**3-15**), all-*cis* acids with five double bonds are represented by clupadonic (7,10,13,16,19-docosapentaenoic) acid (**3-16**) of the *n*-3 series.



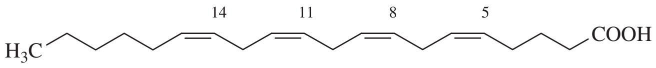
3-12, γ-linolenic acid



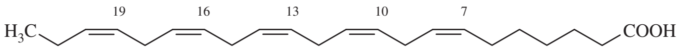
3-13, α-calendic acid



3-14, punicic acid



3-15, arachidonic acid



3-16, clupadonic acid

***n*-6 Fatty acids**

linoleic
(18:2 Δ9.12)
↓ Δ^6-desaturase
γ-linolenic
(18:3 Δ6,9,12)
↓ elongase
dihomo-γ-linolenic
(20:3 Δ8,11,14)
↓ Δ^5-desaturase
arachidonic
(20:4 Δ5,8,11,14)

***n*-3 Fatty acids**

α-linolenic
18:3 Δ9,12,15)
↓ Δ^6-desaturase
stearidonic
(18:4 Δ6,9,12,15)
↓ elongase
eicosatetraenoic
(20:4 8,11,14,17)
Δ^5-desaturase
eicosapentaenoic (EPA)
(20:5 Δ5,8,11,14,17)
↓ elongasa
docosapentaenoic (DPA)
(22:5 Δ7,10,13,16,19)
↓ elongase
tetracosapentaenoic
(24:5 Δ9,12,15,18,21)
↓ Δ^6-desaturase
tetracosahexaenoic
(24:6 Δ6,9,12,15,18,21)
↓ β-oxidation
docosahexaenoic (DHA)
(22:6 Δ4,7,10,13,16,19)

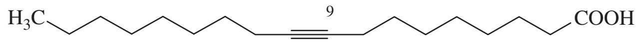
Figure 3.1 Biosynthesis of higher essential fatty acids.

3.3.1.4 Alkynoic, branched-chain and cyclic fatty acids

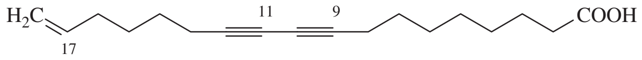
Compared with the above types of fatty acids, these fatty acids are substantially less important in the food industry and in human nutrition.

3.3.1.4.1 Alkynoic acids

Fatty acids with one or more triple bonds (alkynoic, ethynoic or acetylenic acids), or fatty acids containing both double and triple bonds, occur rarely in nature. Examples are stearolic (octadec-9-ynoic) acid (**3-17**) and isanic (octadec-17-en-9,11-diynoic) acid, also known as bolecic acid (**3-18**), in short 9*a*-18:1 (or 18:1, 9*a*) and 17,9*a*,1*a*-18:3 (18:3, 17*E*,9*a*,11*a*). The positional isomer of stearolic acid is tariric (octadec-6-ynoic) acid.



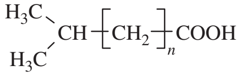
3-17, stearolic acid



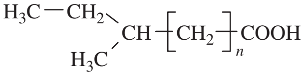
3-18, isanic acid

3.3.1.4.2 Branched acids

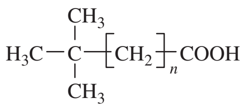
There is a large number of fatty acids (usually saturated) that have a side chain consisting of one carbon, which are therefore methyl derivatives of fatty acids. The methyl group is usually bound to the penultimate carbon atom (*n*–1). These acids are generally referred to as **isoacids** (**3-19**). If the methyl group is bound to the third carbon atom from the end (*n*–2), these fatty acids are called **anteisoacids** (**3-20**). Rare **neoacids** have two methyl groups on the penultimate carbon atom; therefore their terminal group is a tertiary butyl group (**3-21**). An example is 13,13-dimethyltetradecanoic acid (**3-22**). More rarely methyl groups are bound in the middle of the hydrocarbon chain and some fatty acids may have a higher number of methyl group. Typical examples are (4*S*)-4-methyloctanoic (4-methylcaprylic or hircinoic) and (3*RS*,7*R*,11*R*,15)-3,7,11,15-tetramethylhexadecanoic (phytanic) acids (**3-23**). Further examples are presented in Table 3.4.



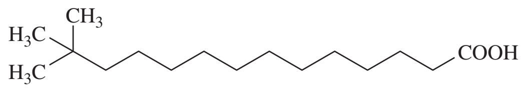
3-19, isoacids



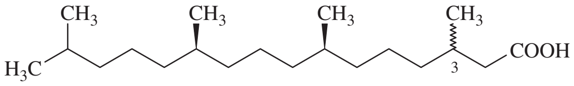
3-20, anteisoacids



3-21, neoacids



3-22, 13,13-dimethyltetradecanoic acid



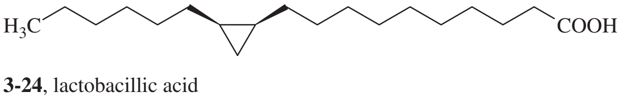
3-23, phytanic acid

Table 3.4 Fatty acids with branched hydrocarbon chain.

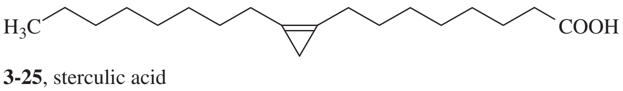
Fatty acid	Number of carbon atoms	Trivial name
3-Methylbutanoic	5	Isovaleric
2,2-Dimethylpropanoic	5	Pivalic
4-Methylpentanoic	6	Isocapric
8-Methylnonanoic	10	Isocapric
10-Methylundecanoic	12	Isolauric
12-Methyltridecanoic	14	Isomyristic
12-Methyltetradecanoic	15	Isopentadecanoic (sarcinic)
14-Methylpentadecanoic	16	Isopalmitic
13,13-Dimethyltetradecanoic	16	Neopalmitic
14-Methylhexadecanoic	17	Anteisoheptadecanoic (methylpalmitic)
15-Methylhexadecanoic	17	Isoheptadecanoic (methylpalmitic)
16-Methylheptadecanoic	18	Isostearic
10-Methyloctadecanoic	19	Tuberculostearic
16-Methyloctadecanoic	19	Anteisononadecanoic (methylstearic)
17-Methyloctadecanoic	19	Isononadecanoic (methylstearic)
2,6,10,14-Tetramethylpentadecanoic	19	Pristanic
3,7,11,15-Tetramethylhexadecanoic	20	Phytanic
3,12,19-Trimethyltricosanoic	26	Phthioic
2,4,6-Trimethyloctacosanoic	31	Mycoceranic

3.3.1.4.3 Cyclic acids

Another group consists of cyclic fatty acids with saturated or unsaturated three-membered, five-membered and six-membered rings. The naturally occurring acids usually have the cyclopropane (**3-24**) or cyclopropene (**3-25**) ring in the middle of the hydrocarbon chain. The cyclopentene ring (**3-26**), as is the case of chaulmoogric

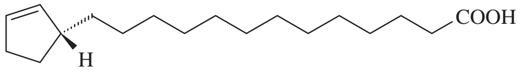


3-24, lactobacillic acid

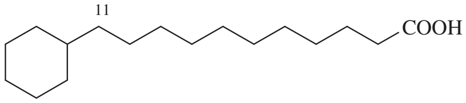


3-25, sterculic acid

acid, is usually at the end of the chain (Table 3.5). An example of fatty acids with the cyclohexane ring at the end of the hydrocarbon chain is 11-cyclohexylundecanoic acid (**3-27**).



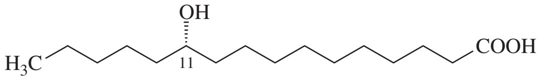
3-26, chaulmoogric acid



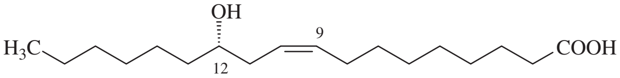
3-27, 11-cyclohexylundecanoic acid

3.3.1.5 Fatty acids with additional oxygen functional group

Hydroxy acids are found naturally in relatively large numbers, but mostly as minor fatty acids. An example of a saturated hydroxy acid is (+)-(*S*)-11-hydroxyhexadeconoic acid, known as jalapinolic acid (**3-28**). The unsaturated hydroxy acid (9*Z*,12*S*)-12-hydroxyoctadec-9-enoic acid, or ricinoleic acid (**3-29**), is the best known representative of the series of (+)-D-acids. It is accompanied by 9,10-dihydroxy- and 9,10,12-trihydroxystearic acids, which can also be found in some edible fats as secondary oxidation products.



3-28, jalapinolic acid



3-29, ricinoleic acid

Important polyhydroxy acids are 9,10,12,13-tetrahydroxyoctadecanoic acids, known as sativic acids (several steric isomers exist), which are derived from linoleic acid. Oxidation of linolenic acid analogously produces 9,10,12,13,15,16-hexahydroxyoctadecanoic (linusic) acids. An overview of common hydroxy fatty acids is given in Table 3.6.

An oxo group (keto group) occurs, for example, in (9*Z*,11*E*,13*E*)-4-oxooctadeca-9,11,13-trienoic acid (**3-30**), which is known under the trivial name α-licanic (couepinic) acid. An example of an epoxy fatty acid derived from ethylene oxide (oxirane) is (*Z*)-11-(3-pentyloxiranyl)undec-9-enoic acid, also known as (*Z*)-12,13-epoxyoctadec-9-enoic acid or 12,13-epoxyoleic acid, by the trivial name vernolic acid (**3-31**).

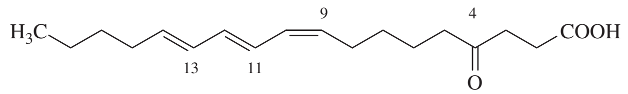
In addition, furan fatty acids can be found in natural products, and are known as F-acids. Their general structure is given in formulae **3-32**. The basic member of the homologous series of non-olefinic furan fatty acids is (10*Z*,12*Z*)-10,13-epoxy-11,12-dimethyloctadecanoic acid (abbreviated as F1-acid; $\omega = 8$, $n = 4$,

Table 3.5 Fatty acids with cyclic residue in chain.

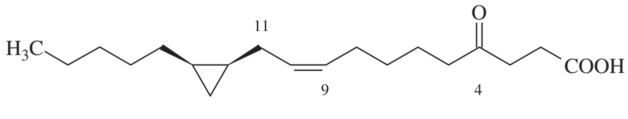
Systematic name	Number of carbon atoms	Number of double bonds	Trivial name
(5*R*)-5-(Cyclopent-2-en-1-yl)pentanoic	10	1	Aleprestic
(1*R*,2*S*,2*Z*)-2-(3-Oxo-2-pent-2-en-1-ylcyclopentyl)ethanoic	12	1	Jasmonic
(7*R*)-7-(Cyclopent-2-en-1-yl)heptanoic	12	1	Aleprylic
(9*R*)-9-(Cyclopent-2-en-1-yl)nonanoic	14	1	Alepric
(11*R*)-11-(2-Cyclopent-2-en-1-yl)undecanoic	16	1	Hydnocarpic
(13*R*)-13-(2-Cyclopent-2-en-1-yl)tridecanoic	18	1	Chaulmoogric
(*Z*)-7-(Octylcycloprop-2-en-1-yl)heptanoic	18	1	Malvalic
(15*R*,9*Z*)-15-(Cyclopent-2-en-1-yl)pentadec-9-enoic	18	2	Hormelic
(13*R*,6*Z*)-13-(Cyclopent-2-en-1-yl)tridec-6-enoic	18	2	Gorlic
(*Z*)-8-(Octylcycloprop-2-en-1-yl)octanoic	19	1	Sterculic
(9*R*,10*S*)-8-(2-Octylcyclopropyl)octanoic	19	1	Dihydrosterculic
(11*R*,12*S*)-10-(2-Hexylcyclopropyl)decanoic	19	0	Lactobacillic (phytomonic)

Table 3.6 Some important hydroxy acids, oxoacids and epoxy acids occurring in lipids.

Fatty acid	Number of carbon atoms	Positions of double bonds	Positions of functional groups	Trivial name
Hydroxy acid				
Hydroxydocosanoic	22		2	Fellonic
Hydroxytetracosanoic	24		2	Hydroxynervonic
Hydroxyhexadecenoic	16	7	16	Ambrettolic
Hydroxyoctadecenoic	18	9	12	Ricinoleic
Hydroxyoctadecenoic	18	12	9	Isoricinoleic
Hydroxytetracosenoic	24	15	2	Cerebronic
Hydroxyoctadecadienoic	18	9, 15	12	Densipolic
Hydroxyoctadecadienoic	18	10, 12	9	Dimorphecolic
Hydroxyoctadecatrienoic	18	9, 12, 13	2	Cemolenic
Hydroxyoctadecatrienoic	18	9, 11, 15	18	Hydroxylinolenic
Dihydroxyoctadecanoic	18	–	9, 10	Dihydroxystearic
Dihydroxytriacontanoic	30	–	9, 10	Lanoceric
Tetrahydroxyoctadecanoic	18	–	9, 10, 12, 13	Sativic
Hexahydroxyoctadecanoic	18	–	9, 10, 12, 13, 15, 16	Linusic
Oxoacid				
Oxooctadecatrienoic	18	9, 11, 13	4	Licanic
Oxooctadecatetraenoic	18	9, 11, 13, 15	4	Parinaric
Epoxy acid				
Epoxyoctadecenoic	18	9	12, 13	Vernolic
Epoxyoctadecenoic	18	12	9, 10	Coronaric
Epoxyoctadecadienoic	18	9, 12	15, 16	Epoxylinoleic



3-30, α-licanic acid



3-31, vernolic acid

$R = CH_3$). The most common compound is (12*Z*,14*Z*)-12,15-epoxy-13,14-dimethyleikosa-12,14-dienoic acid (abbreviated as F1-acid, $\omega = 7$, $n = 3$, $R = CH_3$). Common F-acids are also olefinic fatty acids with one unsaturation on the side chains conjugated with the furan ring. The most common compound is (12*Z*,14*Z*)-12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (abbreviated as F6-acid, $\omega = 7$, $n = 3$, $R = CH_3$).

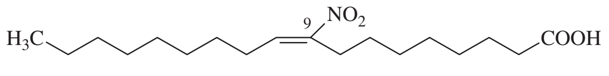
3.3.1.6 Other fatty acids

In addition to the above mentioned fatty acids, fatty acids containing nitrogen, sulfur and chlorine in their molecules also exist in nature. An example of a fatty acid containing a nitro group is 9-nitrooleic acid (**3-33**). An example of a fatty acid containing sulfur in the molecule is 5-(1,2-dithiolan-3-yl)pentanoic acid, which is better known as (*R*)-lipoic or α-lipoic acid (see Section 5.15). A representative of fatty acids with thiophene, tetrahydrothiophene and tetrahydrothiopyran rings in the hydrocarbon chain is 8-(5-hexyltetrahydrothiophen-2-yl)octanoic acid (**3-34**, R = H). Fatty acids with one or more chlorine atoms in the molecule are found in foods as contaminants (see Section 11.4.2.2.2).

3.3.2 Biochemistry, physiology and nutrition

3.3.2.1 Biosynthesis of fatty acids

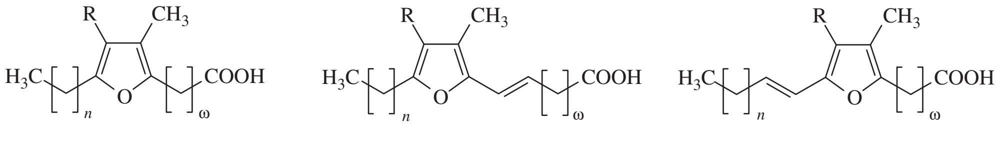
Fatty acid biosynthesis *de novo* is characteristic of all living organisms. The biosynthetic process involves the breakdown of excess dietary carbohydrates into acetate units. Saturated fatty acids are built primarily from acetyl coenzyme A (acetyl-CoA) units in the so-called acetate pathway, with chain assembly being achieved by a Claisen condensation. Acetyl-CoA (connecting glycolysis with the citric acid cycle) is formed from coenzyme A (HS-CoA) and the glycolytic product pyruvic acid. During each cycle, the fatty acid chain is extended by two carbon atoms and the resulting poly-β-oxo



3-33, 9-nitrooleic acid

chain, $-[CH_2-C(=O)]_n-$, is reduced before connecting another molecule of acetyl-CoA. Stabilisation of the chain by cyclisation and partial reduction leads to aromatic polyketides. Therefore, the fatty acids with an even number of carbon atoms are present in lipids far more often than fatty acids with an odd number of carbon atoms. Biosynthesis stops at 16–18 carbon atoms,[3] but in plants producing wax, fatty acid biosynthesis proceeds further. Fatty acids with an odd number of carbon atoms are formed by incorporation of propionyl-CoA instead of acetyl-CoA or by the loss of one carbon atom through α-oxidation. In the sebaceous glands of mammals and birds, malonyl-CoA can be replaced with

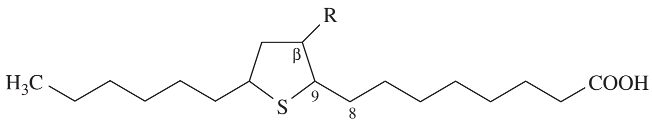
[3]In animal cells and yeasts, multienzyme complexes localised in cytosol, referred to as type I fatty acid synthase (FAS I), carry out the bulk of the *de novo* fatty acid synthesis. In animals, it occurs primarily in the liver, adipose tissue, central nervous system and lactating mammary gland. FAS I contains seven distinct catalytic centres and is arranged around a central acyl carrier protein (ACP) containing bound pantothenic acid (see Section 5.9.1). In prokaryotes and plants, distinct soluble enzymes localised in mitochondria and plastids, referred to as type II fatty acid synthase (FAS II), carry out the reactions.
In the first reaction, acetyl-CoA is converted into acetyl-ACP and partly carboxylated to malonyl-CoA that is transformed into malonyl-ACP by reaction with acetyl-ACP. Condensation of acetyl-ACP with malonyl-ACP followed by decarboxylation gives acetoacetyl-ACP, its reduction yields β-hydroxybutyryl-ACP, dehydration gives (*E*)-but-2-en-1-yl-ACP and on further reduction butyryl-ACP. Butyryl-ACP (with four carbon atoms) reacts with another molecule of malonyl-ACP and each turn of the cycle extends the chain length of the acyl group by two carbons. The acyl group length gradually increases up to 16 carbons giving rise to palmitoyl-ACP. Palmitoyl-ACP can react with malonyl-ACP yielding stearoyl-ACP. Both acyl-ACPs can release the bound fatty acids (palmitic and stearic) or may be transformed (by reaction with HS-CoA) into acyl-CoAs employed in the biosynthesis of lipids (reaction of acyl-CoA with glycerol phosphate).
Palmitoyl-ACP and stearoyl-ACP are precursors for the biosynthesis of unsaturated fatty acids palmitoleic and oleic catalysed by Δ^9-desaturase. Under the action of Δ^4-desaturase, palmitic acid is transformed into (*Z*)-hexadec-4-enoic acid, which gives rise to petroselinic acid by chain elongation. Chain elongation of palmitoleic acid gives asclepic (*cis*-vaccenic) acid. Oleic acid is a precursor of gondic acid (through the action of elongase), which yields erucic acid (elongase) and other fatty acids. The action of Δ^6-desaturase converts oleic acid into (6*Z*,9*Z*)-octadeca-6,9-dienoic acid in mammals, which gives (5*Z*,8*Z*,11*Z*)-eicosa-5,8,11-trienoic acid (Mead's acid) by elongation and dehydrogenation catalysed by Δ^5-desaturase. Mammals cannot synthesise linoleic and linolenic acids, which are only biosynthesised in plants and fungi. Plants and fungi produce linoleic acid from oleic acid by the action of Δ^{12}-desaturase, while Δ^{15}-desaturase catalyses the biosynthesis of linolenic acid. Desaturation of oleic acid on C-9 provides octadec-9-ynoic (stearolic) acid.



non-olefinic furan fatty acids

olefinic furan fatty acids

3-32, furan fatty acids



3-34, 8-(5-hexyltetrahydrothiophen-2-yl)octanoic acid, R = H

methylmalonyl-CoA, which leads to the biosynthesis of methyl derivatives of fatty acids. Thus, 2,4,6,8-tetramethyldecanoic acid is produced from an acetyl-CoA precursor and four methylmalonyl-CoA chain extender units. It occurs, for example, in the sebaceous glands of the goose, producing an oily and waxy secretion, called sebum, to lubricate and waterproof the feathers.

3.3.2.2 Metabolism of essential fatty acids

In addition to fatty acids obtained in the diet, the human organism is able to synthesise some saturated and unsaturated fatty acid in the same way as other animals and plants do. Unlike plants, however, polyene fatty acids of *n*-6 (linoleic acid) and *n*-3 series (linolenic acid) cannot be synthesised although they are strictly necessary. Therefore, these so-called essential fatty acids must be obtained through food in sufficient quantities. In the body, linoleic acid is extended by two carbon atoms and α-linolenic acids by six carbon atoms in elongation reactions catalysed by elongases. New double bonds are created by so-called desaturation, which is catalysed by desaturases. These reactions lead to fatty acids with 20–24 carbon atoms and from three to six double bonds. The oxygenated derivatives of C_{20} are pivotal signaling molecules in animals. In the bodies of animals, these essential fatty acids have an indispensable role as precursors of biologically active compounds called **eicosanoids**.[4] In animal systems, eicosanoids regulate cell differentiation, immune responses and homeostasis. In human metabolism, the most important substance is arachidonic acid (Figure 3.1), which is stored in biological membranes as the C-2 ester of phosphatidylinositol and other phospholipids (see Section 3.5.1). In terrestrial plants, the biological role of eicosanoids is played by other biologically active substances, derivatives of C_{18} and C_{16} fatty acids, known as **octadecanoids** and **hexadecanoids** that act as developmental or defense hormones. The oxygenated derivatives of fatty acids are collectively known as **oxylipins**.

[4]In addition to erythrocytes, **eicosanoids** are produced by all mammalian cells. Eicosanoids include **prostaglandins**, **leucotrienes**, **prostacyclins**, **thromboxanes** and **lipoxins**. These compounds act, for example, as vasoconstrictor and vasodilatory substances in regulating blood pressure, in blood clotting as platelet (thrombocytes) aggregation substances, in regulating leukocyte function, in sleep and waking cycles and other processes. Arachidonic acid yields prostaglandins, prostacyclins and thromboxanes of the series 2 (such as prostaglandin E_2 and thromboxane A_2) *via* cyclic endoperoxides formed by the action of cyclooxygenases (prostaglandin-endoperoxide cyclases) and other enzymes. Leucotrienes are created by 5-, 12- or 15-lipoxygenases. For example, 5-lipoxygenases synthesise Leucotrienes with index 4 and 12/15-lipoxygenases synthesise lipoxins with index 4 from arachidonic acid. Analogously, prostaglandins, prostacyclins and thromboxanes with index 3 and Leucotrienes with index 5 are formed from EPA. Dihomo-γ-linolenic acid is a precursor of prostaglandins with index 1 and of Leucotrienes with index 3.

Enzymes catalysing the desaturation and elongation of *n*-6 and *n*-3 fatty acids are the same, but elongation and desaturation takes place more easily in *n*-3 fatty acids. Some people have less active Δ^6-desaturase, so these transformations are more difficult. The main factors that negatively affect the activity of Δ^6-desaturase are age (in the elderly the enzyme activity is lower), nutrition (ethanol has an inhibitory effect, and there are negative impacts resulting from deficiencies of vitamin B_6, biotin, Zn, Mg and Ca, a higher intake of *trans*-unsaturated fatty acids and positional isomers of unsaturated acids), stress and viral infections. Today, various products containing γ-linolenic acid, dihomo-γ-linolenic acid, eicosapentaenoic acid (EPA) and other substances are available as dietary supplements.

3.3.2.3 Nutrition

The human diet contains only small amounts of free fatty acids. Dietary fat is mostly composed of neutral triacylglycerols accompanied by polar phospholipids and other fatty acid esters, which cannot be absorbed by the intestine. Digestion and absorption of fat requires that the complex fat molecules are broken down enzymatically into smaller, more manageable molecules (see Section 3.4.3.2). Dietary fat should constitute 30–35% of a person's total energy intake, and should contain saturated, monoenoic and polyenoic fatty acids in a ratio of $<1:1.4:>0.6$. Saturated acids should contribute <10%, polyenoic acids of the *n*-6 series 4–8% (on average about 5%) and acids of the *n*-3 series about 1% of energy provided by food. It is often stated that the ratio *n*-6 : *n*-3 should be no greater than 5 : 1. At least 0.5% of the energy intake should come from EPA, DHA and other higher polyunsaturated acids of the *n*-3 series (Figure 3.1).

3.3.2.4 Oxidation in organism

Fatty acids yield energy through a multi step process called β-oxidation, which takes place in the mitochondria of most body tissues. Less common is α-oxidation, which splits the carboxyl group and the chain is shortened by one carbon atom, while generating a fatty acid with an odd number of carbon atoms. ω-Oxidation (oxidation of the methyl group at the end of a chain) can also occur, leading to the formation of dicarboxylic acid.

Fatty acids oxidised during the process of β-oxidation have to be activated by coenzyme A with the formation a fatty acyl-CoA thioester (short and medium length fatty acids undergo this reaction in the mitochondria, while long chain fatty acids form acyl-CoAs at the outer mitochondrial membrane and are carried across the inner mitochondrial membrane by carnitine). The saturated fatty acid molecule with no double bond is then degraded to acetyl-CoA, its chain is shortened by two carbon atoms[5] and the shortened fatty

[5]The fatty acid bound to coenzyme A is first transformed into *trans*-unsaturated (alk-2-enoic) acid, then into 3-hydroxycarboxylic acid and finally into 3-oxocarboxylic acid. This acid breaks down, which results in the production of one acetyl-CoA and a fatty acid shortened by two carbons from each cycle of fatty acid β-oxidation. This acetyl-CoA then enters the mitochondrial citric acid cycle.

acid goes through another β-oxidation cycle. The final reaction product of saturated fatty acids with an even number of carbon atoms is acetyl-CoA. The end product of fatty acids with an odd number of carbon atoms is propionyl-CoA, which has to eliminate carbon dioxide with the formation of D-methylmalonyl-CoA. This is then is isomerised to L-methylmalonyl-CoA, which is rearranged to succinyl-CoA ready to enter the citric acid cycle.

β-Oxidation of unsaturated fatty acids with one *cis*-double bond takes place via similar reactions, but requires two additional enzymes. The β-oxidation cycles proceed as many times as they can before coming to the double bond. Then enoyl-CoA isomerase transforms the *cis*-double bond into a *trans*-double bond and moves it over one carbon so that the product can then continue through the β-oxidation. β-Oxidation of polyunsaturated fatty acids proceeds analogously, but it only goes through one more round using 2,4-dienoyl-CoA reductase to produce a *trans*-3-enoyl product, which is converted by enoyl-CoA isomerase into *trans*-2-enoyl-CoA. Fatty acids with *trans*-double bonds and hydroxy acids are difficult to break down and are a burden for the organism if they are present in large quantities in the diet. *Trans*-unsaturated fatty acids also adversely affect the ratio of low and high density lipoproteins (LDL/HDL) in serum (see Section 3.6.2) and incorporate instead of saturated fatty acids into the *sn*-1 position of the membrane and nervous tissue phospholipids. *Trans*-isomers of fatty acids deteriorate the insulin resistance and thus increase the risk of the metabolic disorder *diabetes mellitus* type 2. Certain pro-inflammatory and adverse effects on fetal development have also been described. Recommendations of the WHO/FAO therefore limit the intake of *trans*-unsaturated fatty acids to 1% of energy intake.

3.3.3 Occurrence

Free fatty acids formed by hydrolysis of lipids under the catalytic action of hydrolases are only found in plant and animal organisms in small quantities. Most of them are bound as esters or amides in homolipids and heterolipids. Some fatty acids, such as palmitic or oleic acids, are common constituents of lipids of all natural materials. Other fatty acids are specific only to microorganisms, plants or animals, some only to certain genera, families or orders, and therefore have particular importance in the taxonomy. For simplicity, the occurrence of free fatty acids and fatty acids bound in lipids is described collectively.

3.3.3.1 Saturated acids

An overview of the occurrence of saturated fatty acids in fats and oils is given in Table 3.7. Generally, the most common fatty acid is palmitic acid, which occurs in virtually all animal and plant lipids, bound in triacylglycerols and phospholipids.

Saturated short chain fatty acids, such as butyric and caproic acids, and the group of medium chain fatty acids acids with 8 and 10 carbons in the molecule typically occur in milk fat (Table 3.8). Butyric acid constitutes 4–8% (being found exclusively at the *sn*-3 position of triacylglycerols), and caproic acid 1–3% (also found mostly at the *sn*-3 position of triacylglycerols) of the weight of all fatty acids in cows' milk fat. These fatty acids are also present in milk fat triacylglycerols of other ruminants, such as sheep and goats, but they are not found in the fats of ruminants other than in the milk fats. Caproic acid is also a minor component of some seed fats (e.g. of palm seeds), which mainly contain fatty acids with medium chain length.

Medium chain fatty acids (C_8–C_{12}) are present, usually as minor components, in milk fat triacylglycerols (Table 3.8). They are not found in the majority of vegetable oils, with a few exceptions. A high content of these fatty acids, especially of lauric acid, which is accompanied by other acids, is found in palm seed oils (of the palm family, Arecaceae), such as coconut and palm kernel oil (Table 3.9) and seed oils of the Laurel family of plants (Lauraceae). Coconut

Table 3.7 Saturated, mono- and polyenoic fatty acids contents of some fats and oil (% of total fatty acids).

Type of fat	Fatty acids			Type of fat	Fatty acids		
	Saturated	Monoenoic	Polyenoic		Saturated	Monoenoic	Polyenoic
Beef tallow	47–86	40–60	1–5	Olive oil	8–26	54–87	4–22
Pork lard	25–70	37–68	4–18	Rice oil	19–35	42–50	16–37
Chicken lard	27–30	42–47	20–24	Cotton seed oil	24–33	15–23	46–59
Milk fat	53–72	26–42	2–6	Wheat germ oil	12–24	24–42	40–62
Carp fat	22–25	46–50	23–28	Soybean oil	14–20	18–26	55–68
Cod liver oil	14–25	35–68	20–45	Sunflower oil	9–17	13–41	42–74
Herring oil	17–29	36–77	10–24	Sesame oil	13–18	36–44	42–48
Coconut oil	88–94	5–9	1–2	Safflower oil	7–13	8–23	68–84
Palm kernel oil	75–86	12–20	2–4	Peanut oil	14–28	40–68	15–45
Palm oil	44–56	36–42	9–13	Rapeseed oil	5–10	52–76	22–40
Cocoa butter	58–65	33–36	2–4	Linseed oil	10–12	18–22	66–72

Table 3.8 Composition of major fatty acids of milk fat (% of total fatty acids).

Fatty acids	Cows' milk	Breast milk	Fatty acids	Cows' milk	Breast milk
Butyric	2.8–4.0	4–8	Σ saturated acids	61	38
Caproic	1.4–3.0	1–4	Σ isoacids	1	0.20.6
Caprylic	0.5–1.7	2–4	Σ anteisoacids	0.8	0.2
Caprinic	1.7–3.2	2–6	Σ branched acids	1.8	0.4
Lauric	2.2–4.5	4–9	Σ *trans*-C18:1 isomers	6.3	0.5
Myristic	5.4–14.6	8–14	Σ *trans*-C20:1 isomers	0.1	traces
Palmitic	26–41	18–35	Σ *trans*-monounsaturated	6.8	-
Stearic	6.1–12.1	7–15	Σ *cis*-C16:1 isomers	1.9	2.2
Arachic	0.95–2.4	0–1	Σ *cis*-C18:1 isomers	23	38
Oleic	18.7–33.4	18–28	Σ *cis*-monounsaturated	26	41
Linoleic	0.9–3.7	2.0–5.2	Σ polyunsaturated	3.5	20
Linolenic	0.1–1.4	0.1–1.1	Σ C18:2 isomers	3.0	17
Arachidonic	0.8–3.0	0.4–1.5	Conjugated linoleic acid	0.7	0.4

Table 3.9 Composition of major fatty acids of the fat of palm seeds (% of total fatty acids; the range of values and average value are reported).

Fatty acid	Coconut oil[a]	Palm kernel oil[b]	Babassu oil[c]	Palm oil[d]	Palmolein	Palmstearin
Caproic	0–0.6	0.0–0.8/0.5	-	-	-	-
Caprylic	4.6–9.4/8	2.4–6.2/4	2.6–7.3	-	-	-
Capric	5.5–8.0/6	2.6–5.0/4	1.2–7.6	-	-	-
Lauric	43.0–51.0/47	41.0–55.0/47	40.0–55.0	0–0.4	0.1–0.2	0.1–0.6
Myristic	16.0–21.0/18	14–18.0/16	11.0–27.0	0.5–2.0/1	0.9–1.4	1.1–1.9
Palmitic	7.5–10.0/9	6.5–10.0/9	5.2–11.0	40–47/45	39.5–41.7	47.2–73.8
Palmitoleic	-	-	-	0–0.6	0–0.4	0.05–0.2
Stearic	2.0–4.0/2.5	1.3–3.0/2.5	1.8–7.4	3.5–6/4.5	4.0–4.8	4.4–5.6
Oleic	5.4–8.1/7	12–19/15	9.0–20.0	36–44 /38	40.7–43.9	16.6–37.0
Linoleic	1.0–2.5/2	1.0–3.5/2.5	1.4–6.6	6.5–12.0/10	10.4–13.4	3.2–9.8
Linolenic	0.0–0.2	0.0–0.7	0	0–0.5	0.1–0.6	0.1–0.6
Arachic	0.0–0.2	0.0–0.3	0	0–1.0	0.2–0.5	0.1–0.6
Eikosenic	0.0–0.2	0.0–0.5	0	0.1	-	-

[a] From the kernel of coconut palm seed (*Cocos nucifera*, Arecaceae).
[b] From the fleshy mesocarp of the fruit of the oil palm (*Elaeis guineensis*).
[c] Derived from the kernel of the fruit of several varieties of the palm *Attalea* spp.; in Brazil from Babassu palm kernels (*Attalea speciosa*). In Mexico and Honduras, related cohune oil is pressed from the endosperm of cohune palm seeds (*A. cohune*).
[d] From the pulp (mesokarp) of oil palm fruits.

oil is derived from the seeds (kernels) of coconuts. Fat extracted from the pulp (mesocarp) of the oil palm is palm oil, which has a completely different composition from palm kernel oil, the fat derived from the kernel (endosperm). Its fractions (palmolein and palmstearin) are commercially available, and are often added to food products.

Myristic acid (**3-1**) is a common lipid component of most living organisms. It usually represents about 1–2% of total fatty acids and is present at higher levels in milk fat (10%) and coconut and palm kernel oils (14–21%). The fat of farm animals, especially of pigs and ruminants, contains long chain saturated fatty acids, palmitic and stearic acids. The content of these fatty acids is lower in the depot fat of commercially reared birds (Table 3.10).

Palmitic acid (**3-2**) is the most common saturated fatty acid. Its content in cow's milk ranges from 26 to 41% and in breast milk from 6.1 to 12.1%. Most animal tissue lipids contain 20–30% palmitic acid; its content in vegetable oils from seeds is 5–30%, while in palm oil it reaches 40% or more. Stearic acid (**3-3**) is found in the highest amount in ruminant fats (milk fat in Table 3.8, and tallow in Table 3.10). In vegetable fats, its highest levels are found in cocoa butter and shea butter (Table 3.11) and then in shortenings. In gangliosides, stearic acid may constitute up to 80% of the total fatty acids. Arachidic acid and other very long chain fatty acids (behenic and lignoceric) are usually present in traces in animal fats; only in some oils do they occur in relatively high concentrations. For example, peanut oil contains 5–7% of C_{20}–C_{24} acids, of which arachidic acid represents about a third. Around 2% of behenic acids are found in canola oil. These and higher fatty acids are found in larger quantities in waxes.

Saturated fatty acids with an odd number of carbon atoms (C_{13}–C_{25}) are relatively rare, and occur only as trace components in triacylglycerols, for example in the milk of mammals. These acids (mainly pentadecanoic acid and heptadecanoic acid, also known as margaric or daturic acid) are present at higher concentrations in the lipids of some microorganisms, in skin lipids and in depot fat and the milk of ruminants (5% or more) from which they enter into the human depot fat.

Table 3.11 Fatty acid composition of plant butters (% of total fatty acids).

Fatty acid	Cocoa butter	Shea butter	Illipé butter
Lauric	-	0.4	0.2
Myristic	0.1	0.3	0.3
Palmitic	23-30	4-8	23
Palmitoleic	0.1-0.4	-	0.2
Stearic	30-36	36-41	23
Oleic	33-36	45-50	34
Linoleic	1-4	4-8	14
Linolenic	0-0.5	0-0.4	0.2
Arachidic	0.2-1.0	1.2	0.2

3.3.3.2 Unsaturated acids

Animal fats and vegetable oils mostly contain unsaturated straight chain fatty acids with 10–36 carbon atoms. The most common fatty acids are monoenoic and polyenoic fatty acids with 16–18 carbon atoms. The unsaturated fatty acids content of fats and oils ranges widely, from more than 90% of total fatty acids in rapeseed oil to less than 10% in coconut oil. Unsaturated fatty acids in animal fats occur in a much smaller concentration range, usually between 50 and 70% (Table 3.7). The only exception is fish oil, because it contains fatty acids with 20–22 carbon atoms and 4–6 double bonds (Table 3.12). The fat of freshwater fish differs in composition from the fatty acids in the fat of marine fish. Fish do not synthesise

Table 3.10 Composition of major fatty acids in the fat of farm animals and birds (% of total fatty acids).

Fatty acid	Beef tallow	Sheep tallow	Pork lard	Rabbit lard	Chicken lard	Goose lard	Emu lard[a]
Lauric	1.0	0.8	traces	traces	0.1	-	-
Myristic	1.4-7.8	2-5	0.5-2.5	4	0.9	0.5	0.3
Palmitic	17-37	20-27	20-32	32	22	21	20
Palmitoleic	0.7-8.8	1.4-4.5	1.7-5.0	6	6	3	3
Stearic	6-40	23-34	5-24	8	6	6	1
Oleic	26-50	30-42	35-62	29	37	54	51
Linoleic	0.5-5.0	1.9-2.4	3-16	19	20	10	14
Linolenic	<2.5	0.6	<1.5	2	1	0.5	1
Arachidic	<0.5	1	<1.0	-	-	-	0.2
Eicosenoic	<0.5	0.2	<1.0	-	1	0.1	0.5

[a] *Dromaius novaehollandiae* (Casuariidae).

Table 3.12 Composition of major fatty acids of fish oils (% of total fatty acids).

Fatty acid	Herring oil[a]	Menhaden oil[b]	Pilchard oil[c]	Cod liver oil[d]
Myristoic	3–10	6–12	4–12	3–5
Myristoleic	–	0.2–0.4	–	–
Pentadecanoic	–	–	–	0.3–0.5
Palmitoic	13–25	14–23	9–22	10–14
Palmitoleic	5–8	7–15	6–13	6–12
Hexadecadienoic	–	3–6	–	0.5–1.6
Stearic	1–4	2–4	2–7	1.0–4.0
Oleic	9–22	6–16	7–17	19–27
Linoleic	1–2	1–2	1–3	1.0–2.0
Linolenic	0.6–2	1–2	0.4–1	0.2–1.0
Octadecatetraenoic	1–5	1–5	2–3	0.4–2.0
Eicosenoic	9–15	0.5–2	1–8	7–15
Eicosadienoic	0.5–0.7	–	–	0.1–0.4
Arachidonic	0.3–0.5	1–4	1–3	–
Eicosapentaenoic	–	12–18	9–35	8–14
Docosenoic	12–27	0.2–0.4	–	4–13
Docosadienoic	0.4–1	–	–	–
Docosatetraenoic	–	–	1–3	–
Docosapentaenoic	0.5–1.3	2–4	1–4	1.1–3.8
Docosahexaenoic	4–10	4–15	4–13	6–17

[a] Atlantic herring (*Clupea harengus*; the herring family Clupeidae).
[b] Atlantic menhaden (*Brevoortia tyrannus*; Clupeidae).
[c] European pilchard (*Sardina pilchardus*; Clupeidae).
[d] Atlantic cod (*Gadus morhua*; Gadidae).

these fats themselves, but acquire them through their food (they are present in plankton, such as crustaceans and algae). Therefore, aquatic mammals (such as whales) that feed on small crustaceans have a similar fatty acids composition to that of fish. There is far greater diversity in the composition of unsaturated fatty acids in plants than in animals.

Vegetable fats and oils are divided into groups according to their related fatty acid composition. Information about the fat content of major vegetable oil raw materials is listed in Tables 3.9, 3.13, 3.14 and 3.15. The following groups are recognised:

- Oils from palm seeds, which generally contain a small amount of oleic acid and only traces of acids with more double bonds (Table 3.9); typical examples are coconut oil and palm kernel oil.

- Plant butters,[6] where the content of unsaturated fatty acids is similar to that in animal fats and consists mainly of oleic acid (Table 3.11 and Table 3.14); a typical example is cocoa butter (today there are objections to the term plant butter as it might be confused with cows' butter).

- Oils with predominantly oleic acid and small amounts of polyunsaturated fatty acids; the most common representative

[6]The name plant butter is derived from the fact that in countries of origin these fats have a consistency like cow's butter, in our climate they are hard fats, but melt in the mouth. A typical example is cocoa butter from cacao beans (*Theobroma cacao*, Sterculiaceae). This is obtained by pressing and is used unrefined in the chocolate and pharmaceutical industries. Because it is expensive, it is often replaced by other plant butters. The most common is shea butter from the African plant commonly known as shea tree or karité (*Butyrospermum parkii*, Sapotaceae, syn. *B. paradoxa*), butter illipe of an Indian plant (*Madhuca longifolia*, syn. *Bassia longifolia* from the same family of plants), butter mowrah from a similar plant (*Madhuca latifolia*), Borneo tallow from the East Asian plant *Shorea stenoptera* (Dipterocarpaceae), Chinese vegetable tallow from the plant *Stillingia sebifera* (Euphorbiaceae), for its rigid consistency often regarded as a wax, and nutmeg (*Myristica fragrans*, Myristicaceae) fat and fat from plants related to nutmeg. Plant butters have a specific composition of triacylglycerols with palmitic, stearic and oleic acid as the main components, which causes a narrow range of melting points of these fats. Sometimes, plant butters are replaced with artificial fat prepared by fractionation of palm oils.

Table 3.13 Composition of major fatty acids of vegetable oils that have a predominance of oleic acid (% of total fatty acids; range of values and average value are reported).

Fatty acid	Olive oil	Almond oil	Hazelnut oil	Avocado oil	Sunflower oil	Peanut oil	Safflower oil
Myristic	0.0–0.1	–	–	–	–	–	<0.1
Palmitic	7.5–20/11.5	4–13	5.1–7.2	9–18	3–5	7	3–6
Palmitoleic	0.3–3.5/1.5	0.2–0.6	0.1–0.3	3–9	<0.2	–	<0.2
Stearic	0.5–5.0/2.5	2–10	1.5–2.4	0.4–1.0	3–5	3	1.5–5.0
Oleic	55–83/74	43–60	71.9–84.0	56–74	70–87	76	74–82
Linoleic	3.5–21.0/9.5	20–34	5.7–22.2	10–17	3–20	4	7–18
Linolenic	0–1.5/1	0	0.0–0.2	0–2	0	0	<0.2
Arachidic	0–0.8	0.1–0.5	–	–	–	2	<1.0
Eicosenoic	0	0.0–0.3	0.1–0.3	–	–	2	<0.2
Behenic	0–0.2	–	–	–	–	4	<0.2
Lignoceric	0–1.0	–	–	–	–	2	–

Table 3.14 Composition of major fatty acids of oils with moderate to high content of linoleic acid (% of total fatty acids; range of values and average value are reported).

Fatty acid	Peanut oil	Sesame oil	Maize germ oil	Sunflower oil	Safflower oil	Cottonseed oil	Poppyseed oil
Lauric	0.0–0.1	0	0.0–0.3	0.0–0.1	0	0.0–0.2	0
Myristic	0.0–0.1	0.0–0.1	0.1–0.3	0.0–0.2	0.0–0.2	0.6–1.0	0.1–0.7
Palmitic	8.3–14.0/10	7.9–10.2/8.5	10.7–16.5	5–8/6.5	5.3–8.0	21.4–26.4/25	7–11
Palmitoleic	0.0–0.2	0.1–0.2	0.0–0.3	<0.5	0.0–0.2	0.1–1.2	0.8–1.6
Stearic	1.9–4.4/3.5	3.5–6.1/4.5	1.6–3.3	2.5–7.0/5	1.9–2.9	2.1–3.3	1–4
Oleic	36.4–67.1/59	35–50/42	24.6–42.2	13–40/24	8.4–21.3	14.7–21.7/18	16–30
Linoleic	14.0–43.0/20	35–50/44	39.4–60.4	40–74/63	67.8–83.2	46.7–58.2/52	62–73
Linolenic	0.0–0.1	0.3–0.4	0.7–1.3	pod 0.3	0.0–0.1	0.0–0.4	–
Arachidic	1.1–1.7	0.3–0.6	0.3–0.6	pod 0.5	0.2–0.4	0.2–0.5	–
Eicosenoic	0.7–1.7	0.0–0.3	0.2–0.4	pod 0.5	0.1–0.3	0.0–0.1	–
Behenic	2.1–4.4	0.0–0.3	0.1–0.5	0.5–1.0	0.2–0.8	0.1–0.6	–
Docosenoic	0.0–0.3	0	0–0.1	0.0–0.2	0.0–1.8	0.0–0.3	–
Lignoceric	1.1–2.2	0.1–0.3	0.1–0.4	0.2–0.3	0.0–0.2	0.0–0.1	–

is olive oil; today, a number of plants containing seeds with oils high in linoleic acid have been bred so that the fatty acid composition resembles the composition of olive oil; examples include sunflower, safflower and peanut oils (Tables 3.13 and 3.14); palm oil can also be included as it contains about 10% linoleic acid and has a high content of palmitic acid (Table 3.9).

- Oils with a medium content of linoleic acid, but not containing linolenic acid; these oils include the traditional peanut oil (also known as arachis oil or groundnut oil) (Table 3.14).

- Oils high in linoleic acid, but not containing linolenic acid, such as traditional sunflower, cottonseed, safflower, poppy seed, germ or sesame oils (Table 3.14).

Table 3.15 Composition of major fatty acids of oils containing linolenic acid (% of total fatty acids; range of values and average value reported).

Mastná kyselina	Rapeseed oil (traditional)[b]	Rapeseed oil (low-erucic acid)[a]	Mustardseed oil	Crambe oil	Soybean oil	Wheat germ oil	Linseed oil
Lauric	0.1	0	Traces	Traces	0.0-0.1	-	-
Myristic	0.2	0.0-0.2	0-1	Traces	0.0-0.2	-	-
Palmitic	1.5-6	3.3-6.0/4.5	0.5-4.5	2	8.0-13.3/10	12-19	4-7/6.5
Palmitoleic	0-3	0.1-0.6	0.0-0.5	0.3	0.0-0.2	0.3-0.5	-
Stearic	0.5-3.1	1.1-2.5/1.5	0.5-2	1	2.4-5.4/4	0.3-3.0	2-5 /3.5
Oleic	8-60	52.0-66.9/56	8-23	12-15	17.7-25.1/21	14-23	12-34/18
Linoleic	11-23	16.1-24.8/21	10-24	8-10	49.8-57.1 /56	50-56	7-27/14
Linolenic	5-13	6.4-14.1/10	6-18	6-7	5.5-9.5/8	3.5-7.0	35-65/52
Arachidic	0-3	0.2-0.8	0-1.5	1-2	0.1-0.6	0.3	-
Eicosenoic	3-15	0.1-3.4	5-13	3-4	0.0-0.3	0.3	-
Eicosadienoic	0-1	0.0-0.1	0-1	-	-	-	-
Behenic	0-2	0.1-0.5	0.2-2.5	0.2	0.3-0.7	0.1	-
Docosenoic[b]	5-60	0-2.0	22-50	55-60	0-0.3	0.3	-
Docosadienoic	0-2	0.0-0.1	0-1	1	-	-	-
Lignoceric	0-2	0.0-0.2	0-0.5	-	0.1-0.4	0.0-0.1	-
Tetracosenoic	0-3	0.1-0.4	0.5-2.5	-	0	-	

[a]Traditional rapeseed oil (turnip rape oil, colza oil, ravison oil, sarson oil or toria oil) is produced from seeds of *Brassica napus*, *Brassica campestris*, *Brassica juncea* and *Brassica tournefortii*. Low erucic acid rapeseed oil (low erucic acid turnip rape oil; low erucic acid colza oil; canola oil) is produced from low erucic acid *Brassica napus*, *Brassica campestris* and *Brassica juncea* seeds.

[b]The maximum permissible concentration of erucic acid in vegetable oils, fats and foods containing more than 5% fat is 5% of total fatty acid content.

- Oils with a medium content of linolenic acid, such as soybean oil and rapeseed 00 oil (Table 3.15); this group also includes oils from seeds of Brassica plants, which originally contained erucic acid[7]; linseed oil is the traditional oil that is rich in linolenic acid.

- Oils containing some specific fatty acids such as γ-linolenic acid (Table 3.16), which occurs in oil from the seeds of evening primrose (*Oenothera biennis*, Oenotheraceae), borage (*Borago officinalis*, Boraginaceae) and seed oil from currants and gooseberries (*Ribes* spp.; Grossulariaceae); erucic acid is found in seed oils of cruciferous plants (Brassicaceae) and plants belonging to the family Tropaeolaceae; petroselinic acid is found in oils of carrot parsley and celery seeds, plants of the Apiaceae family and also in plants of the Araliaceae family.

The most widespread unsaturated fatty acid is oleic acid (**3-4**), which occurs, at least in a small amount, in virtually all animal and plant lipids. Oleic acid constitutes 30–40% of the total fatty acids in adipose tissue of animals and 20–80% of the total fatty acids in vegetable fats and oils. For example, olive oil contains up to 78% oleic acid.

Linoleic acid (**3-7**) is the most common polyenoic fatty acid. It is present, at least in traces, in all fats. For example, sunflower and soybean oils usually contain 50-60% of linoleic acid; safflower oil contains 75% linoleic acid. In the fat of animals, where this essential fatty acid gets from plant food, its content is typically 15-25%, but may be higher (cardiolipin of heart muscle contains 75% linoleic acid). Linolenic acid (**3-11**) is the main component of leaves, especially in the photosynthesising apparatus of algae and higher plants. Linolenic acid is present in linseed oil in amounts of up to 65%. Soybean and rapeseed oils only contain up to 10% linolenic acid. In animal tissues, it is usually a minor component (up to 1%), although the adipose tissue of horses contains up to 10% of this essential fatty acid.

[7]Rapeseed oil traditionally contained a high level of erucic acid (C22:1), which is toxic to humans in large doses. Traditional uses were for lamp oils, soap making, high-temperature lubricating oils and plastics manufacturing. Since 1991, virtually all rapeseed production in the European Union has shifted to rapeseed 00 (double zero oil), with a low content of both erucic acid and glucosinolates, which is a highly appreciated edible oil. The European rapeseed production is conventional (non-GMO). Rapeseed oil has also become the primary feedstock for biodiesel in Europe. Canadian plant breeders produced a premium quality rapeseed oil called canola. The name was derived from '**Can**adian **o**il, **l**ow **a**cid' in 1978. This improvement in the oil resulted from a reduction in erucic acid to levels below 2%.

Table 3.16 Composition of fatty acids of oils for special dietary purposes (% of total fatty acids).

Fatty acids	Evening primrose oil	Borage oil	Blackcurrant oil
Myristic	0.07	0.1	0.1
Palmitic	6-10	9.4-11	6-8
Palmitoleic	0.04	0.4	<0.2
Stearic	1.5-3.5	2.6-5.0	1-2
Oleic	5-12	14.6-21.3	9-13
Linoleic	65-80	36.5-40.1	45-50
α-Linolenic	0.2	0.2	12-15
γ-Linolenic	8-14	17.1-25.4	14-20
Octadecatetraenoic	-	0.2	2-4
Arachidic	0.3	0.2	0.2
Eicosenoic	0.2	2.9-4.1	0.9-1.0
Behenic	0.1	-	0.1
Docosenoic	-	1.8-2.8	-
Lignoceric	0.05	-	0.1
Tetracosenoic	-	1.2-4.5	-

Table 3.17 Content of *trans*-unsaturated fatty acids in some animal fats (% of total *trans*-unsaturated fatty acids).

Double bond position	Milk fat[a]	Butter[a]	Depot fat (beef tallow)[a]
8	1-3	1-2	1-2
9	7-15	5-16	8-14
10	4-13	4-7	5-7
11	28-55	51-68	64-69
12	4-9	3-6	2-3
13	4-9	3-6	2-3
14	4-10	4-7	3-4
15	4-8	3-5	2-3
16	5-10	4-7	3-4

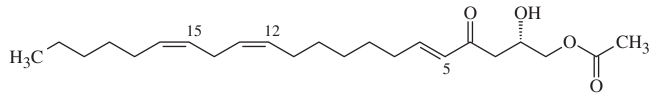
[a]Total amount of *trans*-unsaturated fatty acids in milk fat and butter is 2-8% and in beef tallow it is 2-3% of the total fatty acids.

Milk fat contains some other monoenoic fatty acids, such as caproleic, palmitoleic (about 4%) and asclepic (*cis*-vaccenic, 1–5%; commonly present in vegetable oils, where, as a minor acid, it accompanies oleic acid), and also their *trans*-isomers, especially vaccenic acid (Table 3.17). Larger quantities of myristoleic, palmitoleic (approximately 12–14%) and selacholeic acids are found in fish and whale fats. Palmitoleic acid is present at high levels in the fruits of sea buckthorn (*Hippophae rhamnoides*, Elaeagnaceae) and the oil of macadamia nuts (*Macadamia integrifolia*, Proteaceae). Petroselinic acid, in quantities up to 50% and higher, occurs in the seeds of plants of the Apiaceae family, to which carrots (*Daucus carota*), parsley (*Petroselinum crispum*) and coriander (*Coriandrum sativum*) belong. Ximenic acid is present in the seeds of tropical trees and shrubs of the genus *Ximenia* (Olaceae), from which technical as well as cooking oils are obtained.

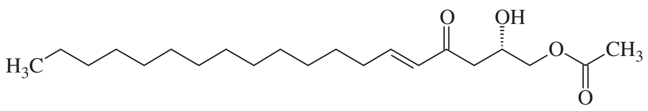
Isomers of linoleic acid with conjugated double bonds occur in milk fat and in lower amounts (2.9–11.3 g/kg) in dairy products and in the meat of ruminants. The main fatty acids are (9*Z*,11*E*)-octadeca-9,11-dienoic (rumenic) acid (**3-10**) and (10*E*,12*Z*)-octadeca-10,12-dienoic acid.

Some conjugated polyenoic fatty acids are also natural components of vegetable oils. α-Calendic acid (**3-13**) is the major fatty acid (50–60%) of oil from the seeds of marigold (*Calendula officinalis*, Asteraceae), and punicic acid (**3-14**) occurs at a level of about 60% in oil from the seeds of pomegranate fruit (*Punica granatum*, Lythraceae). α-Eleostearic acid (with three conjugated double bonds) and β-eleostearic acid are found in significant quantities in tung oil (Chinese wood oil) and abrasin oil, formerly used for lighting and now used for biodiesel production. Tung oil is obtained by pressing the nut of the tung tree (*Vernicia fordii*, Euphorbiaceae), which is native to southern China, Burma and northern Vietnam, but is now grown in subtropical regions around the world. Abrasin oil, used in technological applications similarly to tung oil, is obtained from the *V. montana* tree, and is prized for use in wood finishing. α-Parinaric acid with four conjugated double bonds is present in the seeds of the plant *Atuna racemosa* (syn. *Parinarium laurinum*, Chrysobalanaceae), originally from the island of Fiji and other Pacific islands, where it is present at about 46% (34% of the fatty acids of the oil consists of α-eleostearic acid). α-Parinaric acid is present at about 29% in the seed oil of *Impatiens balsamina* (Balsaminaceae), native to southern Asia and India. Polyenoic acids with isolated double bonds, such as hiragonic (hexadecatrienoic), stearidonic (octacosatetraenoic), clupanodonic (eicosapentaenoic), clupadonic (docosapentaenoic, commonly known as DPA) and nisinic (tetracosahexaenoic) acid, are found primarily in fats of marine fish (Table 3.3). Acetates of unsaturated fatty acids occur as natural substances in avocado fruit (*Persea americana*, Lauraceae). Examples of these fatty acid esters are (2*R*,12*Z*,15*Z*)-2-hydroxy-4-oxoheneicosa-12,15-dien-1-yl acetate, (2*R*,5*E*,12*Z*,15*Z*)-2-hydroxy-4-oxoheneicosa-5,12,15-trien-1-yl acetate, known as persenone A (**3-35**) and (2*R*,5*E*)-2-hydroxy-4-oxononacosa-5-en-1-yl acetate (persenone B, **3-36**), which act as antioxidants, preferentially suppressing radical generation.

Natural fatty acids are mostly of a *cis* (*Z*) configuration. Acids with *trans* (*E*) configuration occur mainly in the depot fat of ruminants



3-35, persenone A



3-36, persenone B

(Table 3.17), resulting from conversion of the digested vegetable fats through biohydrogenation by microorganisms in the rumen, the first and the largest stomach of ruminants. Key products of biohydrogenation are (*E*)-octadec-11-enoic (*trans*-vaccenic) acid (**3-6**), which arises from linoleic or α-linolenic acid and (9*Z*,11*E*)-octadeca-9,11-dienoic (rumenic) acid (**3-10**), which is produced from α-linolenic acid (see Section 3.3.3.1). The ratio of these two fatty acids is about 3 : 1. In the mammary gland, rumenic acid forms via desaturation of *trans*-vaccenic acid.

An increased content of *trans*-unsaturated fatty acids is also found in some other mammals (such as kangaroos) where herbage is pre-digested by microorganisms in their digestive tract. *Trans*-unsaturated fatty acids partly pass into the milk (the predominant acid is vaccenic acid, but also present are elaidic acid, **3-5**, and some other fatty acids). These fatty acids are ingested by humans as a part of their food and therefore occur in human depot fat and in breast milk.

Trans-fatty acids are also formed during industrial catalytic hydrogenation (see Section 3.8.1.7) of plant or fish oils. This hydrogenation improves the thermal stability and prevents oxidation of unsaturated oils. These *trans*-fatty acids therefore occur in fat products based on hydrogenated oils. Unsaturated fatty acids with a *trans*-configuration also form during heating of polyenoic fatty acids (at temperatures over 240 °C); for example, they form during deodorisation of oils (see Section 3.4.3.7). Some invertebrates, but also plants and microorganisms (membrane lipids of bacteria *Pseudomonas putida*), contain traces or even a substantial amount of *trans*-acids.

3.3.3.3 Other acids

3.3.3.3.1 Alkynoic acids

Alkynoic acids are distributed mainly in the lipids of mosses and also in the lipids of some tropical plants. Their occurrence is largely limited to species of plants of the family Santalaceae, where the seeds of some plants of the genera *Exocarpus*, *Santalum* and *Ximenia* contain stearolic acid (**3-17**), plants of the Simaroubaceae family contain tariric acid, and plants of the Olaceae family contain isanic acid (**3-18**). Boleka oil, containing tariric acid, and isano oil, containing isanic acid, are used in technological applications.

Alkynoic fatty acids are toxic. It is assumed that they interfere with the metabolism of lipids and fatty acids and inhibit lipoxygenases and cycloxygenases.

3.3.3.3.2 Branched acids

The basic member of the homologous series of branched chain carboxylic acids is 3-methylbutanoic (isovaleric) acid, which is ranked among the hemiterpenoids (see Section 8.2). Triacylglycerols containing isovaleric acid are important components of fat in European sturgeon, known as beluga (*Huso huso*), a species of anadromous fish in the sturgeon family (Acipenseridae). Isovaleric acid occurs in larger amounts in valerian root (*Valeriana officinalis*, Valerianaceae), in the form of esters of various monoterpenoids (such as borneol, also known as bornan-2-ol) and some less common sesquiterpenoids.

Higher isoacids and anteisoacids are predominantly found in the depot fat and milk of ruminants (Table 3.18) at levels of 1–2%. The dietary branch chain fatty acids come from milk, dairy products and meat of ruminants. They also constitute the lipid part of cell walls in some colon bacteria. Several mechanisms appear to be involved in their formation. Some branched chain fatty acids arise from acetyl-CoA elongation of 2-oxoacids acting as the precursors of amino acids (such as valine and isoleucine), without the involvement of fatty acid synthase mediated reactions, suggesting integration of amino acid and fatty acid metabolism. Methyl side chains can also be introduced when methylmalonyl-CoA replaces malonyl-CoA as the chain-extending unit in fatty acid biosynthesis (see Section 3.3.2.1).

Common branched chain fatty acids are anteisoheptadecanoic acid (14Me 16:0), anteisononadecanoic acid (16Me 18:0), anteisopentadecanoic acid (12Me 14:0) and anteisotridecanoic (10Me 12:0) acid. These acids occur in higher levels in the lipids of mammal hairs. Lanolin (wool fat) for example, contains isoacids and anteisoacids C_{10} to C_{34}. One of these, the anteisoacid 18-methyleicosanoic acid, represents up to 60% of fatty acids bound by thioester bonds in sheep wool. Fish oils contain 1–2% C_{14}–C_{18} isoacids and anteisoacids. 4-Methyloctanoic (4-methylcaprylic or hircinoic) and 4-methylnonanoic acids have been implicated as primary contributors to the so-called 'soo' odor of mutton, which is an unpleasant fatty-goaty type note. The odour recognition threshold of (*S*)-hircinoic acid is 13 μg/l in air.

Microbial degradation of isoprenoids in the rumen of ruminants, for example of the chlorophyll side chain formed by (2*E*,3,7*R*,11*R*,15)-3,7,11,15-tetramethylhexadec-2-en-1-ol (phytol), yields some more branched acids, such as (2*RS*,6*R*,10*R*)-tetramethylpentadecanoic (pristanic) or (3*RS*,7R,11R,15)-3,7,11,

Table 3.18 Content of branched chain fatty acids in animal fats (% of total fatty acids).

Number of carbon atoms	Milk fat (cow)	Beef tallow	Sheep tallow	Pork lard
13	0.06	traces	traces	0
14	0.05	<0.3	0.1	0
15	0.77	<1.5	1.2	<0.1
16	traces	<0.5	0.5	<0.1
17	0.42	<0.5	1.6	traces
18	traces	traces	0.2	0

15-tetramethylhexadecanoic (phytanic) acid (**3-23**). Phytanic acid is therefore found in dairy products such as butter, and in the fat of ruminants,[8] as a mixture of (3*RS*)-epimers. Branched fatty acids obtained by oxidation of paraffins are used for technical purposes, for example in cosmetics. Branched fatty acids are less utilisable.

Plant isoacids and anteisoacids are the main component of the surface waxes. For example, pine seeds (*Pinus* sp., Pinaceae) contain 14-methylhexadecanoic acid at a level of 0.5–1%. A higher number of branched acids is a typical characteristic of lipids in the cell walls of certain bacteria (including pathogens). An example is (*R*)-tuberculostearic acids of *Mycobacterium tuberculosis* and related species. Neoacids occur in some microorganisms, algae, higher plants and marine animals. For example, 13,13-dimethyltetradecanoic acid (**3-16**) is a minor component of conifer resins.

3.3.3.3.3 Alicyclic acids

Alicyclic saturated fatty acids with a cyclopropane ring and alicyclic unsaturated fatty acids with a cyclopropene ring are relatively rare in natural fats and oils. These fatty acids are found at trace levels in the lipids of bacteria and lipids of other lower organisms and plants. An example is *cis*-11,12-methyleneoctadecanoic acid, known as lactobacillic or phytomonic acid (**3-24**). It was first isolated as a constituent of triacylglycerols from the bacteria *Lactobacillus arabinosus*, and was later found in many other bacteria. This acid is accompanied by *cis*-9,10-methylenehexadecanoic acid and C_{18}–C_{20} homologues. Some microorganisms contain *cis*-9,10-methylenoctadecanoic (dehydrosterculic) acid.

Sterculic acid (**3-25**) occurs at low concentrations (1.0–2.3 g/kg) in cottonseed (*Gossypium* spp., Malvaceae) oil accompanied by malvalic acid (2.4–3.4 g/kg) and dihydrosterculic acid (1.0–1.8 g/kg). In unroasted cottonseed meal, concentrations of these acids are about ten times lower. This fatty acid is found in a significantly higher amounts in seed oils of the genus *Sterculia* plants in the mallow family (Malvaceae), originating in tropical South America, where it is also accompanied by lower homologues, malvalic and dihydrosterculic acids. Fruits known as tropical chestnuts have a flavour reminiscent of pistachios. The sterculic acid and malvalic acid contents in oils from *S. foetida* are about 49 and 6%, respectively, and in *S. monosperma* about 19 and 0.6%. These oils have special properties because they polymerise at normal temperatures and at elevated temperatures (around 250 °C) they form gels. Gum karaya is extracted from *Sterculia* species and is used as a thickener and emulsifier in foods. Dihydrosterculic acid is found at a level of 35–48% in the oil from the seeds of Chinese lychee (*Litchi chinensis*) and longan fruits (*Dimocarpus longan*) of the Sapindaceae family.

Cyclic unsaturated fatty acids with a cyclopentene ring at the end of the chain, such as chaulmoogric acid (**3-26**) and hydnocarpic acid, are present in the seeds of evergreen trees known as chaulmoogra (*Hydnocarpus*, syn. *Taraktogenos*) of the Achariaceae (formerly Flacourtiaceae) family, which grow in the rain forests of Southeast Asia. Poisonous fruits of *H. wightiana* contain about 35% of oil with a specific odour that was used in folk medicine for the treatment of leprosy and skin diseases.[9] The oil of related species *H. anthelminticus* contains about 68% chaulmoogric, 9% hydnocarpic and 1% gorlic acids. The content of these acids in the oil of *H. kurzii* is about 35, 23 and 23%, respectively.

Fatty acids with a cyclohexane ring at the end of the hydrocarbon chain, such as 11-cyclohexylundecanoic (**3-27**) and 13-cyclohexyltridecanoic acids are minor constituents of milk, butter, sheep's tallow and rumen bacteria. The amount of 11-cyclohexylundecanoic acid in cow and goat milk is 1–2 g/kg. It was found that this acid has inhibitory effects on the bacteria *Bacillus cereus*, *Escherichia coli* and fungi *Fusarium culmorum*. Cyclic fatty acids are also found in the ordinary oils, where their formation is induced by heating (see Section 3.8.1.5).

3.3.3.3.4 Epoxy acids

Epoxy fatty acids occur either as 1,2-epoxy compounds derived from ethylene oxide or 1,4-epoxides derived from furan (furan acids).

Natural 1,2-epoxides are saturated and unsaturated compounds with 18 carbon atoms, which are found mainly in small amounts as components of cutin and oils from plant seeds. Vernolic acid (**3-31**) occurs in the seeds of trees of the genus *Vernonia* of the Asteraceae family, originating from tropical Asia and America. For example, seeds of *V. anthelmintica* and of *V. galamensis* contain 62–78% of vernolic acid. Coronaric acid is found at levels of 8–18% in the seeds of garland, also known as chrysanthemum greens or edible chrysanthemum (*Chrysanthemum coronarium*) from the same plant family. The leaves of these plants are often eaten as a salad. Small amounts of 9,10-epoxystearic (about 2.5% of total fatty acids), vernolic and coronaric acids occur in germ peanut oil (*Arachis hypogaea*, Fabaceae).

Furan fatty acids (**3-32**) are present in plants, fishes, amphibians, reptiles and mammals, including man. In some fish, F-acids can typically represent 1–6% of the fatty acids in the liver lipids. In some freshwater fish, however, furan fatty acids may represent up to 25% of the total fatty acids. They also occur in butter, meat, vegetable oils, shrimps and mushrooms in small amounts. For example, virgin olive oils contain non-olefinic 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid ($\omega = 8$, $n = 4$, $R = CH_3$) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid ($\omega = 10$, $n = 4$, $R = CH_3$) and two olefinic derivatives: 12,15-epoxy-13,14-dimethyleicosa-12,14,16-eicosatrienoic acid ($\omega = 10$, $n = 2$, $R = CH_3$) and 12,15-epoxy-13,14-dimethyleicosa-10,12,14-eicosatrienoic acid ($\omega = 8$, $n = 4$, $R = CH_3$). Furan fatty acids also arise as products of lipid oxidation. Autoxidation or photoxidation of furan fatty acids yields α,β-unsaturated γ-lactones known as bovolides (see Figure 8.54).

[8] Phytanic acid is normally present in small amounts in human tissues. Many defects in the α-oxidation pathway, including Refsum's disease, result in an accumulation of phytanic acid leading to neurological distress, deterioration of vision, deafness, loss of coordination and eventually death.

[9] Compounds responsible for this specific odour, in addition to other components, are the degradation products of cyanogenic glycoside gynocardin with a cyclopentene ring (see Section 10.3.2.3.1).

3.3.3.3.5 Hydroxy acids

2-Hydroxy fatty acids with 16–26 carbon atoms are important components of animal sphingolipids (see Section 3.5) and plant waxes occurring on the surface of leaf vegetables. In addition, 2-hydroxy fatty acids and 3-hydroxy fatty acids are characteristic compounds of the lipid component of the lipopolysaccharides, which are located in the outer membrane of Gram-negative bacteria. For example, even-numbered 2- and 3-hydroxy fatty acids (with chain lengths of from 8 to 16 carbons) were identified in bovine milk fat. 2-Hydroxyoctanoic acid (45 mg/kg) was present in the highest amount, followed by 3-hydroxyhexadecanoic (42 mg/kg), 3-hydroxydodecanoic (34 mg/kg), 3-hydroxydecanoic (32 mg/kg) and 3-hydroxytetradecanoic (25 mg/kg) acids. Other 2- and 3- hydroxy fatty acids were present in lower amounts. In food samples, both (*R*)-enantiomers (D-hydroxy fatty acids) and (*S*)-enantiomers (L-hydroxy fatty acids) of 2- and 3-hydroxy acids were detected, with the (*R*)-enantiomer being the enantiopure or predominant. In particular, 2- and 3-hydroxyhexadecanoic acids were found to contain relevant proportions of the (*S*)-enantiomer.

4-Hydroxycarboxylic acids and 5-hydroxycarboxylic acids occur in the form of corresponding γ- and δ-lactones in many fruits, especially apricots and peaches. Many other hydroxy fatty acids are also found in seed oils of plants. For example, (*S*)-jalapinolic acid (**3-28**) occurs in lipophilic ester-type dimers of acylated pentasaccharides derived from L-rhamnose in sweet potato (*Ipomoea batatas*, Convolvulaceae), which are known as batatins. (9*Z*,12*S*)-12-Hydroxyoctadec-9-enoic (ricinoleic) acid (**3-29**) occurs in castor oil, where it represents about 90% of the total fatty acids. So-called castor oil is extracted from the seeds of the castor oil plant (*Ricinus communis*) of the Euphorbiaceae family, and is used only for technical purposes as it has purgative properties.

3.3.3.3.6 Oxoacids

Oxoacids (ketoacids) are less common than hydroxy acids. Saturated oxoacids with 10–24 carbon atoms and unsaturated oxoacids with 14–18 carbon atoms and the carbonyl group at C-5 to C-13 represent about 1% of fatty acids in milk fat. The so-called oiticica oil is extracted from the seeds of subtropical and tropical South American plants of the genus *Licania* (Chrysobalanaceae), which includes shrubs and trees, such *L. rigida* and *L. arborea*. This oil typically contains licanic acid (**3-30**). Parinaric acid occurs in some plant oils of the rose family (Rosaceae) and the balsam family (Balsaminaceae).

3.3.3.3.7 Other acids

Nitro fatty acids are one of the forms used to link the signaling pathways of the two major groups of cell function regulators: eicosanoids and reactive forms of nitrogen, derived from nitric oxide (NO). Their interaction with the oxidised fatty acids can be crucial in the regulation of pro-oxidative and antioxidative processes in the immune responses and development of inflammatory processes. The most abundant nitro fatty acids are 9- (**3-33**) and 10-nitrooleic and 10- and 12-nitrolinoleic acids. Nitro derivatives of linolenic and icosapentaenoic acids have also been described.

Sulfur-containing fatty acids are rarely encountered. The lower homologue of (*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid, known as (*R*)-lipoic or α-lipoic acid (see Section 5.15), is 1,2-dithiolane-3-carboxylic acid, known as tetranorlipoic or tetranorthiooctic acid. It was recently isolated from garlic (*Allium sativum*, Amaryllidaceae), together with sulfur substituted C_{18} fatty acids with thiophene, tetrahydrothiophene (**3-33**) and tetrahydrothiopyran rings. An example of these fatty acids is 8-(5-hexyltetrahydrothiophen-2-yl)octanoic acid (**3-34**, R = H). A derivative of this fatty acid, with the tetrahydrothiophene ring substituted with a methyl group in the β-position (**3-34**, R = CH_3), and other two position isomers (the corresponding C_{18} hexanoic and heptanoic acids) were found as minor sulfur-bearing components of unprocessed canola oil.

Chlorine-containing fatty acids arise as contaminants in reactions of unsaturated fatty acids with chlorine or chlorine dioxide (see Section 11.4.2.2.2).

3.3.4 Properties of fatty acids

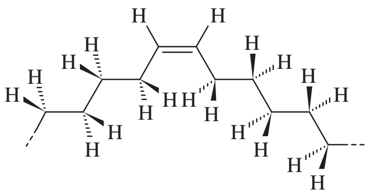
Fatty acids are colourless liquids or solids. Lower saturated fatty acids are liquid, while caprinic (capric) acid and higher fatty acids are solid at room temperature. Their melting points depend on the number of carbon atoms, but when this number is higher than 20, the melting point does not change significantly (Table 3.19).

Fatty acids with an odd number of carbon atoms have a slightly lower melting point than fatty acids with an even number with one less carbon atom. Unsaturated fatty acids with a double bond in the middle of the chain have significantly lower melting points than saturated fatty acids. Considerable influence on the melting point (the conformation of the molecule) comes from the *cis*-double

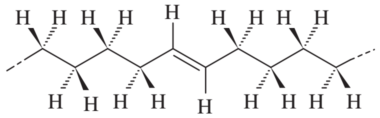
Table 3.19 Melting points of important fatty acids.

Fatty acid	Melting point (°C)	Fatty acid	Melting point (°C)
Saturated		***Unsaturated***	
Butyric	−7.9	Petroselic	33.0
Caproic	−3.4	Oleic	16.0
Caprylic	16.7	Elaidic	45.0
Caprinic	31.6	Asclepic	15.0
Lauric	44.2	Vaccenic	43.8
Myristic	54.1	Linoleic	−5.0
Palmitic	62.7	Linolelaidic	28.5
Stearic	69.6	Linolenic	−10.6
Arachidic	75.4	Erucic	33.5
Behenic	79.9	Brassidic	61.5
Lignoceric	84.2	Arachidonic	−49.5

bond, which causes bending of the straight chain by about 42°; therefore, *cis*-derivatives (**3-37**) have melting points a few tens of degrees lower than the corresponding *trans*-derivatives (**3-38**). The shift of the double bond toward the carboxyl in *cis*-monoenoic acids leads to an increase in the melting point. A higher number of double bonds in the all-*cis* configuration results in lowering of melting points (Table 3.19).

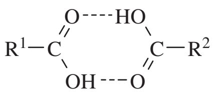


3-37, chain conformation of *cis*-monoenoic acid



3-38, chain conformation of *trans*-monoenoic acid

The majority of common unsaturated fatty acids are viscous thixotropic liquids (see Section 7.8.3.2.2). Thixotropy is the property of certain gels or fluids that are thick (viscous) under normal conditions, but flow (less viscous) over time when agitated or otherwise stressed. As with liquid saturated fatty acids, they are associated by hydrogen bonds of the carboxyl groups and form dimers (**3-39**).



3-39, association of fatty acids by carboxyl groups

Melting point also depends on the crystalline modification of the fatty acid.[10] On cooling, fatty acids pass from the liquid phase first to a solid unstable phase, which is gradually transformed into more stable modifications. Modifications are labelled A, B and C in acids with an even number of carbon atoms, and A′, B′ and C′ in acids with an odd number of carbons in the molecule (Figure 3.2).

Forms A and A′ are triclinic crystals (one of the seven crystal systems described by three basis vectors), other forms are orthorhombic crystals. Fatty acids can generally be present in any form (considering the conditions of crystallisation). Saturated fatty acids usually form the stable modification rather quickly; unsaturated fatty acids are converted more slowly (because e.g. oleic acid has two modifications melting at different temperatures, 14 and 16 °C). The crystals of fatty acids form layers, in which carboxyl groups are oriented to one side and the methyl groups of the chain end to the other side. The chains are parallel to each other (Figure 3.3). This arrangement of fatty acid molecules leads to soft crystals. Single crystal modifications differ by the slope of fatty acids to the plains of individual layers. Even above the melting point, the fatty acids preserve the partially oriented structure, which causes the already mentioned thixotropy of these compounds.

Lower fatty acids are volatile at atmospheric pressure, while higher fatty acids are non-volatile. Their boiling point increases with the increasing number of carbon atoms, but double bonds have little influence. Fatty acids with short carbon chains are miscible with water, while others are basically soluble in water, but solubility decreases rapidly with an increasing number of carbon atoms in the molecule (Table 3.20) and a two-phase system is created. It is also necessary to consider the water solubility in fatty acids, which also rapidly decreases with an increasing number of carbon atoms. Higher fatty acids dissolve in water only slightly, but can form monomolecular films (a closely packed layer of molecules) in which fatty acid molecules are oriented towards each other, with carboxyl groups to the aqueous phase and the methyl groups to the gaseous phase. When reducing the surface area, the monomolecular films collapse, creating a multimolecular layer (see Section 7.6.2.3).

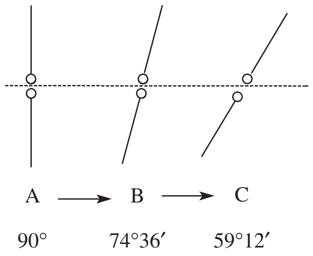


Figure 3.2 Slope of saturated fatty acid chain in different crystal modifications: —— = hydrocarbon chain, o = carboxyl, ------ = crystal plane.

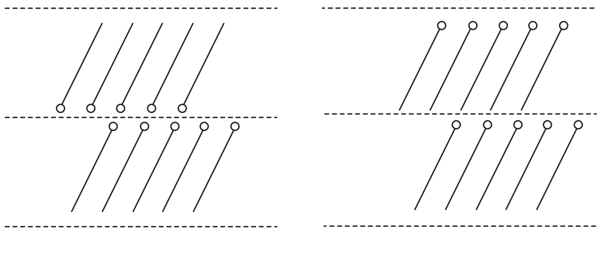


Figure 3.3 Simplified packing patterns of fatty acids in crystals (solid phase).

[10]Solid substances with the same chemical composition and different crystal structure are called polymorphic forms or modifications. Each polymorphic form has characteristic properties (such as specific volume and melting point). The formation of a given polymorphic form depends on many factors (such as temperature, cooling rate of the liquid phase and type of solvent). In the solid crystal state, one polymorphic form is transformed into the other polymorphic forms without previous melting of the crystals. If one of the forms and another form is stable, these two polymorphic forms are called monotropic. The transformation occurs only in one direction, the stable form arises from the metastabile form. All natural fats are monotropic.

Table 3.20 Solubility of some fatty acids at 20 °C.

Fatty acid	Solubility (g/l)		Fatty acid	Solubility (g/l)	
	Water	Ethanol (95%)		Water	Ethanol (95%)
Butyric	Miscible	Miscible	Lauric	0.055	912
Caproic	9.70	Miscible	Palmitic	0.0072	49.3
Caprylic	0.68	Miscible	Stearic	0.0029	11.3

Fatty acids are a group of weak acids. In solutions, they are found almost exclusively in non-dissociated form. Formic and acetic acids are much stronger acids than higher homologues of this series.

3.4 Homolipids

Homolipids, which consist of fatty acids linked to various alcohols, are now categorised exclusively by the structure of the bound alcohol. The most common alcohol in natural lipids is glycerol. Rarely occurring alcohols are glycols, higher monohydric aliphatic alcohols, aliphatic and alicyclic terpenoid hydroxy compounds, such as xanthophylls (see Section 9.9.1.2), and various steroid compounds (see Section 3.7.4).

3.4.1 Esters of monohydric alcohols

Esters of fatty acids with monohydric alcohols are known by the trivial name of a **wax**. The most important natural compounds are fatty acids esters with various:

- aliphatic alcohols, which were previously called **cerides**
- alicyclic alcohols, formerly known as **sterides**.

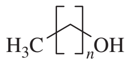
The term wax, however, is used for pure compounds and also for a variety of natural products of animal and vegetable origin, components of which are fatty acid esters with monohydric alcohols, but also other lipophilic compounds, such as higher hydrocarbons, free alcohols, ketones and fatty acid. These compounds, with the exception of fatty acids, are classified as lipid accompanying substances (see Section 3.7). Esters of fatty acids with monohydric alcohols may even be minority constituents in these natural mixtures. It may therefore be more appropriate to use the term wax just for natural products rather than for pure compounds that are often called **wax esters**.

The waxy material on the surface of the above-ground parts of plants, such as the skins of fruit and leaves of vegetables, seeds and pollen grains, is called surface or **epicuticular wax**. The next hydrophobic layer of the cuticle is **cutin**, which is a polymeric material built from hydroxy fatty acids. Wax and cutin together constitute the **epicuticular lipids**. In most plants, wax is not associated with cutin at all. On the surface of the underground parts of plants, wax does not occur. Polymeric lipid material on the surface of the underground parts of plants and on any injured tissues is called **suberin**. However, cutin and suberin are not classified as lipids.[11]

3.4.1.1 Structure and nomenclature

Esterified fatty acids bound in waxes are usually saturated acids with a higher number of carbon atoms (fatty acids with long and ultra long chains having 20–34 carbon atoms). Fatty acids with hydroxyl group often occur, for example, in position C-4. These then easily form δ-lactones, which are then always present in industrial waxes in small amounts.

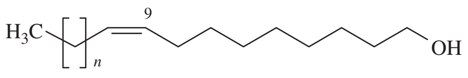
Policosanol is the common name for a mixture of high molecular weight (12–36 carbon atoms) saturated aliphatic primary alcohols, which are constituents of plant epicuticular waxes, such as the common alcohols cetyl alcohol (C_{16}), ceryl alcohol (C_{26}) and myricyl alcohol (C_{30}) (**3-40**). The cuticular wax of wheat, for example, contains lignoceryl alcohol (C_{24}), ceryl alcohol (C_{26})



3-40, lauryl alcohol, n = 11
myristyl alcohol, n = 13
cetyl alcohol, n =15
stearyl alcohol, n = 17
arachinyl alcohol, n = 19
docosyl alcohol, n = 21
lignoceryl (carnaubyl) alcohol, n = 23
ceryl alcohol, n = 25
tetraenanthyl alcohol, n = 27
myricyl (melissyl) alcohol, n = 29
lacceryl alcohol, n = 31
tritriacontan-1-ol, n = 33

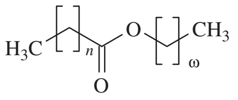
[11]The building units of cutin are called cutin acids, which include mono-, di- and trihydroxycarboxylic fatty acids and also higher dicarboxylic, dihydroxydicarboxylic and also other acids. The characteristic monomers are 18-hydroxyoctadeca-9,12-dienoic, 10,16-dihydroxyhexadecanoic and *threo*-9,10,18-trihydroxyoctadecanoic acids.
Suberin has a qualitatively similar composition, but the amounts of the individual compounds differs. The prevailing acids are C_{16}–C_{22} dicarboxylic acids (such as octadec-9-ene-1,18-dioic acid), C_{20}–C_{26} fatty acids and alcohols, which constitute about one third of the suberin weight. The remainder is composed of phenolic compounds.

and myricyl alcohol (C_{28}) as the major polycosanol components. Myristoleyl alcohol and oleyl alcohol are derived from unsaturated fatty acids (**3-41**). Less frequently, secondary aliphatic alcohols can be found.



3-41, myristoleyl alcohol, *n* = 5
oleyl alcohol, *n* = 7

Alcohols are usually esterified with fatty acids of similar structure. The characteristic structure is expressed by formula **3-42**. The compound name is formed by the radical names of the alcohol and the bound acid. For example, ceryl cerotate is the ester of cerotic acid with ceryl alcohol. The main wax ester on the surface of sunflower seeds is ceryl cerotate. The main surface wax esters of apples and cabbage leaves are ceryl palmitate and ceryl stearate. The constituents of the epicuticular wax of apple fruits are also C_{16}–C_{26} fatty acid esters of (*E*)- and (*Z*)-*p*-coumaryl alcohol. The fatty acid esterification occurs at the γ-hydroxyl group rather than at the 4-hydroxyl on the phenyl ring.



3-42, general structure of aliphatic waxes
cetyl palmitate, *n* = 14, ω =15
ceryl cerotate, *n* = 24, ω = 25
lacceryl laccerate, *n* = 30, ω = 31

Fatty acids can also be bound to various secondary aliphatic alcohols, such as nonacosane-15-ol, to carotenoids containing hydroxyl groups (known trivially as xanthophylls, see Section 9.9.1.2), as well as to flavonoids and some terpenoids (steroids) (see Section 3.7.4). Other wax constituents, in particular the epicuticular wax components, include alkanes, aldehydes, ketones and free fatty acids.

3.4.1.2 Biochemistry, physiology and nutrition

The main significance of waxes is that they form a hydrophobic layer on the surface of organisms. In animals these occur in the skin, hair, fur or feathers, while in plants they are found in the surface layers of leaves, fruits and other aerial (above ground) parts. They act mainly to control transpiration, preventing water loss by evaporation, and protecting the organisms against environmental influences, insects and parasites.

Waxes are synthesised by reduction of fatty acids to primary alcohols via aldehydes. Primary alcohols react with acyl-CoAs to form esters, aldehydes eliminate carbon monoxide (by decarbonylation) giving rise to hydrocarbons (alkanes). Oxidation of alkanes yields secondary alcohols, and oxidation of secondary alcohols gives rise to ketones.

Waxes do not decompose easily in the body, but when they do, the mechanism is similar to that of fatty acids in fats and oils with similar chain lengths. In human nutrition, waxes have virtually no significance.

3.4.1.3 Occurrence

Waxes are widely distributed in nature in both the animal and plant kingdoms, but are usually only seen in foods at low levels.

3.4.1.3.1 Animal waxes

The wax found in the cranial cavity of the marine mammal the sperm whale (*Physeter macrocephalus*, syn. *P. catodon*) and two related species (pygmy sperm whale and dwarf sperm whale) from the sperm whale superfamily has industrial importance. The milky-white waxy substance, or spermaceti, is composed mainly of cetyl palmitate (**3-42**). The proportion of spermaceti with a lower melting point, containing predominantly unsaturated fatty alcohols, is called sperm oil.

Another animal wax is beeswax, which is used by honey bees to build honeycomb cells in which their larvae are raised and honey and pollen are stored. Its major component is ceryl cerotate (**3-42**), but a diverse mix of its homologues is also present. The basic composition of animal waxes is shown in Table. 3.21.

The industrially important animal waxes include the wax on the surface of wool, which is obtained as a by-product of wool scouring, and after bleaching and purifiying this wax is termed lanolin. The alcohol components are mainly triterpenic (alicyclic) alcohols, such as lanosterol (see **3-92**), cholesterol (see **3-114**) and related steroids. Lanolin is used in the manufacture of cosmetics and pharmaceuticals.

3.4.1.3.2 Plant waxes

An example of the composition of cuticular waxes is the cuticular wax of thale cress (also known as mouse-ear cress or Arabidopsis

Table 3.21 Main wax acids and alcohols.

Wax	Fatty acid	Fatty alcohol
Animal		
Beewax	Palmitic, cerotic	Ceryl alcohol
Spermaceti	Palmitic	Cetyl alcohol
Sperm oil	Lauric, myristic	Oleyl alcohol, myristoleyl alcohol
Plant		
Chinese	Cerotic, montanic	Ceryl alcohol
Carnauba (Brasil, palm)	Cerotic	Ceryl alcohol, myricyl alcohol
Jojoba	Eicosenoic, docosenoic	Eicosanol, docosanol
Shellack	Cerotic, lacceric	Ceryl alcohol, lacceryl alcohol

(*Arabidopsis thaliana*, Brassicaceae), which is a plant used as a model organism in molecular biology and genetics. The major components of this wax are alkanes (38%), followed in quantity by ketones (30%), primary alcohols (12%), secondary alcohols (10%), aldehydes (6%), free fatty acids (3%) and their esters (1%). The main alkane is nonacosane (C_{29}), the main ketone is ditetradecylketone (C_{29}), myricyl alcohol (melissyl alcohol, C_{29}) and ceryl alcohol (C_{26}) are the main primary alcohols, the main secondary alcohols are C_{29}, C_{31} and C_{27} compounds, and the major fatty acids are mellisic acid (C_{30}) and montanic acid (C_{28}). The epicuticular wax of wheat and related species contains alkanes (5–10%), esters (10–30%), aldehydes ($\leq$5%), alcohols (15–55%) and acids ($\leq$5%).

The plant waxes that are of industrial importance include a hard, white wax known as Chinese wax, which is secreted by Indian Wax Scale insects (*Ceroplastes ceriferus*, syn. *Coccus ceriferus*) on injury of Chinese ash (*Fraxinus chinensis*, Oleaceae) and laid on tree branches. Japan wax is actually not the true wax, but a pale-yellow fat obtained from the fruits of the sumac wax tree (*Rhus succedanea*, Anacardiaceae) that grows in China and Japan. The seeds of jojoba shrubs (*Simmondsia californica*, Buxaceae), native to the desert of Sonora and adjacent areas of the United States and Mexico, contain a liquid wax (with melting point of 7 °C), which is called jojoba oil (wax). Candelilla wax is a yellowish-brown, hard wax located on the leaves and shoots of the small Candelilla shrub (*Euphorbia antisyphilitica*, Euphorbiaceae) growing in northern Mexico and the southwestern United States. Carnauba wax (also known as Brazil wax or palm wax) is a hard yellow–brown wax secreted on the surface of leaves of *Copernicia cerifera* (Arecaceae) palms from tropical South America. Palm wax similar to the previous one is also obtained from Andean palm *Ceroxylon andicola* and related palm species. Myrica wax comes from the California Wax Myrtle growing in North America (*Myrica cerifera*, Myricaceae). It is used to manufacture candles and perfumes. Similar waxes are produced by other species of the genus *Myrica*. Shellac is a product obtained after removal of dyes from lacks, resinous materials arising from various fig trees (*Ficus* spp., Moraceae), flowering plants of the genus *Butea* (Fabaceae) and other plants after injury caused by the Lac insect (*Coccus lacca*). An overview of fatty acids and alcohols bound in important plant waxes is shown in Table 3.21.

Esters of fatty acids with monohydric alcohols can also be produced by industry or in the laboratory. In this case, however, esters with lower alcohols, namely methanol, ethanol, propan-2-ol or butan-1-ol are produced. Esters of fatty acids with methanol are components of so-called biodiesel; esters of fatty acids with ethanol and fusel oil alcohols are common components of alcoholic beverages.

3.4.1.3.3 Mineral waxes

Some waxes are of mineral origin. They come from the breakdown products of natural plant waxes. These include montan wax (also known as lignite wax) and the refined product ozokerite. Their alcoholic component and bound fatty acids have longer hydrocarbon chains than plant waxes.

3.4.1.4 Properties

Waxes are usually hard materials with melting points between 60 and 110 °C. They are insoluble in water and poorly soluble in organic solvents. Some waxes are liquid and also hydrophobic, but they dissolve well in organic solvents, can leave greasy stains and easily forming emulsions. Esters of lower alcohols produced by industry also have some of the properties of liquid waxes, but due to their lower molecular weights they have a lower viscosity and better solubility in alcohols.

3.4.1.5 Use

In the food industry, waxes are used as agents to repel water (hydrophobicity) on the surface of fruits and other food products, and to improve their appearance (fruits and some sweets). The waxes that are allowed for use in foods are legally limited to white and yellow beeswax, candelilla wax, carnauba wax and shellac.

Spermaceti was historically used primarily for the manufacture of cosmetic products such as ointments, cosmetic creams and pomades. Later it was used for the manufacture of fine wax candles, in textile finishing and as an industrial lubricant. Natural or bleached beeswax is used in the cosmetics and pharmaceutical industries, and a practical uses include the making of candles (today only for luxury and church candles), and various dressings and polishes. Lanolin is an important raw material in the cosmetics and pharmaceutical industries.

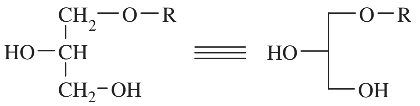
Chinese wax is used in perfumery and skin polishing. Japan wax is used for the manufacture of polishing creams for floors, footwear and furniture and for the manufacture of candles and matches and in the cosmetics industry. Jojoba oil is a liquid; it is therefore used for the production of fat emulsions and moisturisers in the cosmetics and pharmaceutical industries, as a carrier for speciality fragrances and as a lubricant for delicate mechanisms. It has also been considered as a low-energy replacement for edible fats. The wax prepared from the oil is used as a polishing wax, in the manufacture of carbon paper and linoleum. It also has a potential use as a biodiesel fuel.

Candelilla wax and carnauba wax are mainly used for the production of candles. Carnauba wax can produce a glossy finish and as such is used in automobile waxes, shoe polishes and waxing skin. Carnauba wax is also used for the preparation of polishing waxes, carbon paper and many other purposes. Palm wax is the raw material for the production of wax candles and matches. Myrica wax and related waxes have found application in the pharmaceutical and food industries. Shellac is used to make paints, varnishes, sealing waxes, adhesives and in insulating materials.

As waxes are a relatively expensive raw material, in the production of cosmetics and candles they have been replaced by mineral hydrocarbon fractions (paraffin), solid triglycerides (tristearin, also known as stearin) or by various synthetic materials (polyethylene and polypropylene). Mineral waxes are used almost exclusively for non-food purposes. The so-called montanic acid esters and oxidised polyethylene wax can also be used to modify the surfaces of fresh citrus fruits. Montanic acid esters are used pharmaceutically to improve the retardation of drug release from tablets.

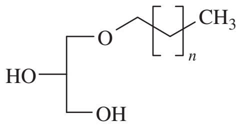
3.4.2 Glyceryl ethers

Monoalk(en)ylethers of glycerol or 1-*O*-alky(en)yl-*sn*-glycerols or 1-alkoxy-*sn*-glycerols (**3-43**) are compounds that have bound fatty alcohol in the *sn*-1 position of the glycerol. They are related to plasmalogenes (see Section 3.5.1.1.2), which are glycerylethers of phospholipids and classified as heterolipids.



3-43, glyceryl ether (glyceryl 1-*O*-alkylether)

The main monoalkylethers of glycerol are 1-*O*-hexadecyl-*sn*-glycerol (chimyl alcohol, **3-44**), 1-*O*-octadecyl-*sn*-glycerol (batyl alcohol, **3-44**) and (*Z*)-1-*O*-octadec-9-en-1-ylglycerol (selachyl alcohol, **3-45**). Trivial names are derived from the names of the animals from which the fats were first isolated. Glyceryl ethers are not hydrolysed by lipases. In nature, glyceryl ethers are mostly found as esters of higher fatty acids. For example, 2-*O*-arachidonoylglyceryl ether derived from arachidonic acid, trivial name noladin ether, occurs in the brain of pigs. The most common compounds are diesters of glyceryl ethers, 1-*O*-alk(en)yl-2,3-diacyl-*sn*-glycerols.



3-44, chimyl alcohol, $n = 14$
batyl alcohol, $n = 16$

Glyceryl ethers are present in animal and plant lipids in amounts that normally do not exceed 1%. They are found at higher levels in microbial lipids and lipids of some marine animals (cartilaginous fish, starfish, urchins and clams), especially in lipids of cartilaginous fishes of the class *Chondrychthyes*, subclass Elasmobranchii, which includes sharks (Selachii) and rays (Batoidea). Shark liver oils commonly contain about 53% triacylglycerols, 45% diesterified glyceryl ethers, 1.5% cholesteryl esters and 0.5% squalene. In the liver oils of some species of these animals, depending on the season, glyceryl ethers can constitute about 10% (in spiny dogfish, *Squalus acanthias*), but also up to 50–80% of the unsaponifiable fraction (in squaliform sharks of the genus *Centrophorus* and in kitefin sharks of the genus *Scymnorhinus*).

3.4.3 Glyceryl esters

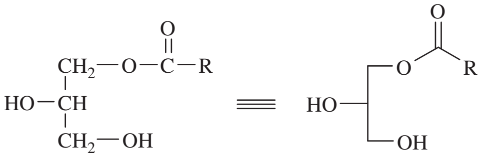
Glyceryl esters are the most important food lipids. They are generally known by the names of their physical state as **fats** or **oils**, even if the name fats should be only be used for natural products containing triacylglycerols that are usually solid at ambient temperature, rather than for individual compounds. If fats are liquid at ambient temperature, they are called oils. In the past vegetable oils were categorised according to the behaviour of a thin film of the oil when exposed to the air. The categories that could be distinguished were:

- **non-drying oils** (such as olive, peanut, coconut, palm, palm kernel and castor oils) that do not harden when exposed to air
- **semi-drying oils** (soybean, sunflower, poppyseed, sesame and cottonseed oils)
- **drying oils** (linseed, safflower and perlila oils; see Section 8.2.1.1.1) that harden after exposure to air.

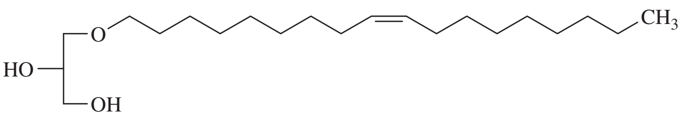
However, these groups do not have precise boundaries. Today, the division of triacylglycerols into fats and oils has only historical significance, and the term 'fat' commonly refers to the entire group, regardless of consistency.

3.4.3.1 Structure and nomenclature

The glycerol molecule can be bound to one, two or three fatty acids. The resulting monoesters are then either 1-**monoacylglycerols** (**3-46**) or 2-monoacylglycerols (**3-47**). These esters were formerly called monoglycerides and this name is often still used, especially among experts from industry. The ratio of both isomers in natural materials depends on the stereospecifity of lipase synthesising monoacylglycerols, and the major compounds in food lipids are the more stable 1-monoacylglycerols (Table 3.22). Two fatty acids bound on the glycerol molecule yield 1,2-**diacylglycerols** (**3-48**) or 1,3-diacylglycerols (**3-49**), which predominate in natural fats. Diacylglycerols were previously called diglycerides. Monoacylglycerols and diacylglycerols are collectively referred to as **partial esters of glycerol**. In nature, the most frequently occurring glyceryl esters contain three fatty acids bound to the glycerol molecule. They are known as **triacylglycerols** (formerly known as triglycerides). Nearly all commercially important fats and oils of animal and plant origin consist almost exclusively of triacylglycerols (Table 3.22).



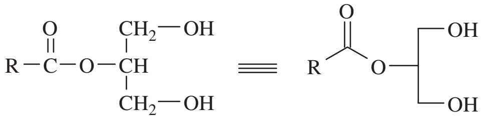
3-46, 1-monoacylglycerol



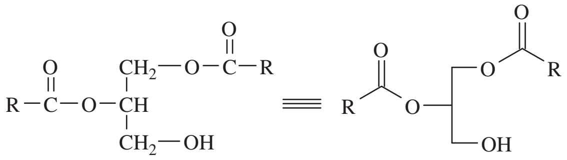
3-45, selachyl alcohol

Table 3.22 Composition of glycerol esters in refined edible oils.

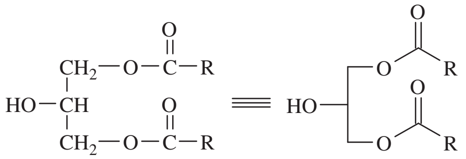
Type of ester	Content (%)	
	Rapeseed oil	Sunflower oil
1-Monoacylglycerols	0.6	0.2
2-Monoacylglycerols	0.1	0.05
1,3-Diacylglycerols	1.9	0.9
1,2-Diacylglycerols	0.2	0.1
Triacylglycerols	96.5	97.8



3-47, 2-monoacylglycerol

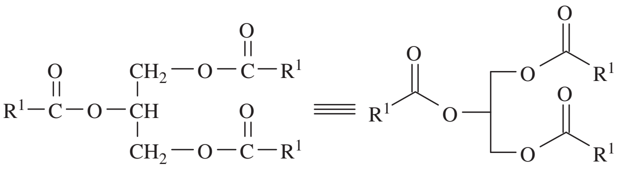


3-48, 1,2-diacylglycerol

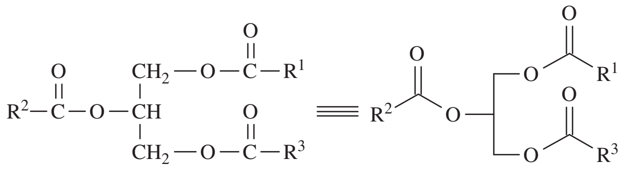


3-49, 1,3-diacylglycerol

All three fatty acids bound in triacylglycerols can be the same, and in this case we may speak about **simple triacylglycerols** (**3-50**). For example, the triacylglycerol with three identical acyls, derived from palmitic acid, is 1,2,3-tripalmitoylglycerol or simply tripalmitoylglycerol, or tripalmitin to give it its trivial name. Three acyl residues derived from oleic acid are found in triolein and three stearic acid residues are found in tristearin. Alternatively, the two or three fatty acids bound in triacylglycerols can be different, and in this case we speak about **mixed triacylglycerols** (**3-51**).

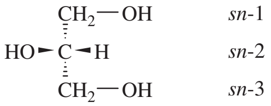


3-50, simple triacylglycerol



3-51, mixed triacylglycerol

For example, 1-palmitoyl-2-stearoyl-3-oleoylglycerol (formerly also known as 1-palmito-2-stearo-3-oleine) is different from 1-palmitoyl-2-oleoyl-3-stearoylglycerol and other positional isomers. These positional isomers lead to great diversity in the composition of natural fats and oils. When the two primary hydroxyl groups (in positions C-1 and C-3) of glycerol are esterified with different fatty acids, the resulting triacylglycerol is asymmetric and optically active and the diversity of composition of natural fats and oils thus increases. Natural fats contain optically active triacylglycerols because they are formed by esterification through the catalytic action of hydrolases that are stereospecific. Since the bound fatty acids have very similar properties, these stereoisomers do not differ significantly in their optical rotations. Nevertheless, an exact definition of the composition of fats should also mention their steric structure. To express the exact composition and stereo configuration of triacylglycerols, the so-called stereo specific numbering system (*sn*-system) is used, as recommended by a IUPAC-IUB commission, which defines the numbering of atoms in prochiral glycerol ('L-glycerol' derivatives) as that shown in formula **3-52**. The conventional D/L or *R*/*S* systems are not used. The secondary hydroxyl group is shown to the left of C-2 and the carbon atom above this then becomes C-1, while that below becomes C-3 and the prefix *sn* is placed before the stem name of the compound. The term triacyl-*sn*-glycerol should then be used instead of triacylglycerol.



3-52, *sn* numbering of glycerol in Fischer projection

Three different fatty acids can occur from 27 different mixed triglycerides. To simplify the description, one- or two-letter abbreviations of fatty acids are used, such as B = butanoic (butyric), D = decanoic (capric or caprinic), H = hexanoic (caproic), L = linoleic, La = lauric, Ln = linolenic, M = myristic, O = oleic, Oc = octanoic (caprylic), P = palmitic, Po = palmitoleic, S = saturated, St = stearic, U = unsaturated and V = vaccenic. For example, the mixed triacylglycerol esterified with palmitic acid in position *sn*-1, with stearic acid in the position *sn*-2 and oleic acid in the position *sn*-3 of glycerol is then called 1-palmitoyl-2-stearoyl-3-oleoyl-*sn*-glycerol (abbreviated to *sn*-PStO). Racemate is a mixture of *sn*-PStO and and *sn*-OStP in a molar ratio of 1 : 1

(or rac-PStO), where the fatty acids in positions *sn*-1 and *sn*-3 are reversed. Triacylglycerol PStO can generally be a mixture of up to six optically active substances.

3.4.3.2 Biochemistry and physiology

3.4.3.2.1 Biosynthesis

Synthesis (lipogenesis) of triacylglycerols occurs primarily in the cytoplasm of the liver, adipose tissue, central nervous system and the lactating mammary gland. The fatty acids are derived from the hydrolysis of fats, as well as from the synthesis of acetyl CoA through the oxidation of fats, glucose and some amino acids. In animals, the liver and intestines are the most active, although most of the body stores of triacylglycerols are in adipocytes, the fat-storing cells of adipose tissue. Two main biosynthetic pathways are known. The most important route is the *sn*-glycerol 3-phosphate pathway (also known as the Kennedy pathway), which predominates in the liver, adipose tissue and skeletal muscle, and the 3-monoacyl-*sn*-glycerol pathway in the intestines. More than 90% of liver triacylglycerols are produced by the *sn*-glycerol 3-phosphate pathway. After a meal, up to 75% of the triacylglycerols are formed in the enterocytes of the intestines, via the 3-monoacyl-*sn*-glycerol pathway. In some animal tissues, a third, less well known biosynthetic pathway has been recognised. In this pathway, triacylglycerols are synthesised by transacylation between two racemic diacylglycerols.

The building block for the biosynthesis of glycerol is the glycolytic product (*R*)-phosphoglyceric acid.[12] In the first step, it is hydrolysed to (*R*)-glyceric acid by glycerate kinase, glyceric acid is oxidised by specific aldehyde dehydrogenase to (*R*)-glyceraldehyde (D-glyceraldehyde) and the reduction is then catalysed by glycerol dehydrogenase to yield glycerol. Glycerol is activated by phosphorylation with glycerol kinase to prochiral *sn*-glycerol 3-phosphate, which is esterified by fatty acid residues in acyl-CoA with catalysis by glycerol 3-phosphate *O*-acyltransferase. The first reaction product is 1-acylglycerol 3-phosphate (3-*sn*-lysophosphatidic) acid, which is esterified to 2-diacylglycerol 3-phosphate (3-*sn*-phosphatidic acid) by 1-acylglycerol 3-phosphate *O*-acyltransferase. Hydrolysis of the phosphate by phosphatidate phosphatase yields 1,2-diacylglycerol. The last esterification of 1,2-diacylglycerol to triacylglycerol is catalysed by diacylglycerol *O*-acyltransferase. Phosphatidic acid also serves as a precursor of glycerophospholipids.

3.4.3.2.2 Fat digestion, absorption, transport and mobilisation

The human fat-digestive enzymes include triacylglycerol lipases and phospholipases. The triacylglycerol digestive enzymes comprise the pre-duodenal lingual, gastric and extra-duodenal pancreatic lipase. Although a small amount of lipase is secreted by the Ebner's glands on the tongue and by the stomach, these digestive actions are not significant in children and adults as almost no real breakdown of fat occurs. However, the stomach is a part of the process of fat digestion because of its churning action, which helps to create an emulsion. The fat enters the first section of the small intestine (duodenum) in the form of gastric chyme and stimulates the secretion of the peptide hormone cholecystokinin synthesised in the mucosal epithelium of the small intestine. The secretion of cholecystokinin causes the gall bladder to release bile acids that further emulsify the fat and release of the colipase-dependent pancreatic lipase by the pancreas, which is activated in the intestinal lumen by trypsin. The hydrolysis by pancreatic lipase results in almost complete hydrolysis of triacylglycerols in the intestinal lumen. Most of the bile acids are returned to the liver for re-use. In newborns, the pancreatic secretion of lipase is low. The digestion of fat is mainly augmented by the lipases secreted from the glands of the tongue (lingual lipase) and by the lipase occurring in human milk.

Lipases catalyse the hydrolysis of dietary triacylglycerols primarily to fatty acids (their sodium or potassium salts) and 2-monoacyl-*sn*-glycerol via 1,2-diacyl-*sn*-glycerol or 2,3-diacyl-*sn*-glycerol. The hydrolytic attack is regiospecific and occurs at the *sn*-1 or *sn*-3 positions, but not at the *sn*-2 position of triacylglycerols. To some extent, 2-monoacylglycerol is partly spontaneously racemised to 1-monoacylglycerols, which may be subsequently completely hydrolysed to fatty acid and glycerol.

Phospholipids are hydrolysed by pancreatic phospholipase A_1 (phosphatidylcholine 1-acylhydrolase) and phospholipase A_2 (phosphatidylcholine 2-acylhydrolase) present as a zymogen that requires activation by trypsin. The products of 1,2-diacyl-*sn*-glycero-3-phosphocholine breakdown are fatty acids and lysophospholipids, that is, 2-acyl-*sn*-glycero-3-phosphocholine and 1-acyl-*sn*-glycero-3-phosphocholine, respectively. Cholesterol esters are hydrolysed by pancreatic steryl ester acylhydrolase (cholesterol ester hydrolase) to give cholesterol and fatty acids.

The digestion products of triacylglycerols retain their association with bile acids and lipase and combine with other lipids to form small aggregates called micelles that are suspended within the ingesta. The micelles bump into the brush border of the enterocytes (columnar epithelial absorptive cells of the intestinal wall) into which the digestion products pass by diffusion.

Glycerol is transported to the liver or kidneys and is either converted into glucose via glycerol 3-phosphate (gluconeogenesis) or used to help the breakdown of glucose into energy (glycolysis). Fatty acids with a chain length of less than 14 carbon atoms are absorbed directly into the blood via intestine capillaries as complexes with albumin, enter into the portal vein (which can also act as an absorptive route for dietary long chain fatty acids) and are transported to the liver. Monoacylglycerols and fatty acids with 14 or more carbons are not released directly into the intestinal capillaries and need to be reesterified to triacylglycerols. The re-esterification proceeds by the 3-monoacyl-*sn*-glycerol pathway that produces more than 75% of postprandial triacylglycerols. In addition to the 3-monoacyl-*sn*-glycerol pathway, the intestine can also synthesise triacylglycerols via the glycerol 3-phosphate pathway,

[12] To a lesser extent, 1,3-dihydroxyacetone phosphate (formed as a product of glycolysis and photosynthesis in plants) is employed for the biosynthesis of glycerol. In the depot tissue (in adipocytes), 1,3-dihydroxyacetone phosphate is the only precursor of triacylglycerols. It can either be reduced to glycerol 3-phosphate by glycerol 3-phosphate dehydrogenase or first acylated to 1-acylglycerol, which is reduced by acylglyceronphosphate reductase to 3-*sn*-lysophosphatidic acid.

which is the dominant triacylglycerol synthetic pathway in other tissues, such as adipose and liver. Beginning in the endoplasmic reticulum, the main site of lipid synthesis, and continuing in the Golgi apparatus (an organelle that is a part of the cellular endomembrane system), triacylglycerols are assembled, along with other compounds, into a lipoprotein particle called chylomicron, which consists of triacylglycerols (85%), phospholipids (6–12%), cholesterol and cholesteryl esters (1–3%) and proteins (apolipoproteins, 1–2%). Chylomicron is transported into the villi, the finger-like projections that cover the walls of the small intestine, and enters the circulation via the lymphatic capillary known as the lacteal, which finally merges into the thoracic duct. The thoracic duct empties the chylomicrons into the bloodstream via the left subclavian vein for transport to tissues where triacylglycerols are stored or metabolised for energy.

In the target tissues, the chylomicron triacylglycerols are hydrolysed by lipoprotein lipase, located on the interior walls of the capillary blood vessels, to fatty acids and 2-monoacylglycerols. Most fatty acids and 2-monoacylglycerols are absorbed by the cells (adipose cells or muscle fibres) and employed for triacylglycerol re-synthesis. A significant proportion of digested fat is typically stored as body fat in the adipocytes found mostly in the abdominal cavity and subcutaneous tissue. Within all cell types, triacylglycerols are stored as cytoplasmic lipid droplets called adiposomes, organelles enclosed by a monolayer of phospholipids and hydrophobic proteins, such as the perilipins in adipose tissue. The remnants of the chylomicron are transported to the liver, hydrolysed and the products absorbed. Fat soluble vitamins (vitamins A, D, E and K) and cholesterol are also delivered to the liver as part of the chylomicron remnants.

Animals also accept fats in the diet in very small amounts through the mechanism of pinocytosis (the process of taking fluid, together with its contents, into the cell by forming narrow channels through its membrane that divide off into vesicles). The bulk of dietary lipids are triacylglycerols that must be enzymatically digested to yield fatty acids and monoacylglycerols.

Triacylglycerols represent a form of energy storage for most organisms (in animal adipose tissue and plant seeds), but also act as membrane components and as signalling molecules. The fat reserves in humans are usually 10–30 kg, which would be sufficient to cover the energy needs for several months. The energy content of lipids is about 38 kJ/g, which is roughly twice the energy content of carbohydrates or proteins of the same weight. During storage in the body they do not bind water, so that this stock of energy does not weigh too much.

If the body needs energy, it supplies triacylglycerols in the form of hydrophilic lipoproteins to the relevant cells, where triacylglycerols are hydrolysed and fatty acids are oxidised, mainly by a series of reactions termed β-oxidation, to produce energy and building blocks for growth. The use of fatty acids for energy production and re-synthesis of triacylglycerols is predominantly performed in the liver. The major source of lipids entering the liver does so in free fatty acid form released from adipose tissue and transported in the systemic blood plasma complexed with albumin. The fatty acids within the liver can be utilised for a variety of purposes, from oxidation (see Section 3.3.2.4) to the synthesis of structural lipids.

3.4.3.3 Nutrition

The human diet contains fats of animal and vegetable origin. Some fats are obvious (such as those added during cooking), but roughly the same amounts are hidden fats already present in the raw materials (mainly of animal origin). Triacylglycerols derived from the diet of the populations in industrialised countries deliver 30–40% of the energy requirements. In developed countries, this energy intake is usually excessive (more than 100% of the recommended amount). In Europe it would be prudent ('it is recommended') to reduce ('to lower') the contribution of fat to total energy intake below 30% to prevent the major public health problems associated with fat inadequate intake (such as the risk of developing high blood pressure, diabetes, cardiovascular diseases and certain forms of cancer). However, the proportion of fat must not fall below 20% of energy supplied, otherwise various disorders may occur, mainly due to an inadequate supply of lipophilic vitamins and essential fatty acids. The proportion of saturated fatty acids in the total energy intake from fat should be less than 10%, the proportion of *trans* fatty acids should be less than 2%, the proportion of polyunsaturated fatty acids of the *n*-6 series should be less than 4–8% and unsaturated fatty acids of the *n*-3 series should correspond to 2 g of linoleic acid and 200 mg of fatty acids with longer chains.

3.4.3.4 Occurrence and composition

3.4.3.4.1 Occurrence

The total content of lipids in common foods is given in Table 3.23. The dietary fats occur almost exclusively as triacylglycerols. However, they may contain 1–10% of the partial esters of glycerol, a smaller amount of phospholipids and about 1% of accompanying compounds. Humans mainly receive fats by eating plant tissues and animal reserve tissues in which they are stored. According to their origin, fats are divided into:

- plant fats
- animal fats
- other fats.

In plants, fats are mainly found in seeds, but also in the pericarp layers, the outside exocarp layer (peel), the middle layer (pith) and the inner endocarp layer. Fat is also present in the germs of seeds, in which starch is the main reserve substance (e.g. the germ of cereals). Fat composition is due mainly to the fatty acid content, but specific properties of lipases also play a role.

Animal fats are further divided into the fats of terrestrial animals (including milk and depot fats) and seafood (including the fat of marine mammals, such as whales, and fish oils). In animal products, it is mainly the subcutaneous adipose tissue that is consumed, but fat is also stored in the muscle and offal, and in the liver of some fish (such as cod).

Fats are also present in microorganisms, higher fungi, algae and other organisms, but these sources are of negligible significance in human nutrition.

Table 3.23 Lipids contents of some common foods.

Foods	Lipid content (%)		Foods	Lipid content (%)	
	Fresh weight	Dry weight		Fresh weight	Dry weight
Foods of animal origin			Bread (white)	0.8–1.1	1.3–1.7
Pork meat (lean)	18	51	Bakery products (white)	2–3	8–12
Pork meat (fatty)	41	75	Confectionary products	14–34	22–44
Beef meat	2–36	9–63	Chocolate	32–38	33–40
Calf meat	3–7	4–10	Fruits	0.2–0.7	1.0–2.8
Poultry (fowl)	1–35	5–50	Leafy vegetables	0.2–1.0	2.8–6.2
Poultry (waterfowl)	17–33	40–65	Root vegetables	0.3–0.4	1.3–1.8
Offal	2–15	8–45	Potatoes	0.2	0.8
Sausages	25–48	60–65	Walnuts	64	66
Fish	0.4–16	2–44	Almonds	54	57
Milk (full fat)	3.8	30	Soybeans	13–20	14–22
Milk (reduced fat)	1.8	12	Beans	1.6	1.8
Milk (condensed)	9.5	12	Pea	1.4	1.6
Milk (dry, full fat)	27	28	Margarine	80–82	98–99
Cream	34	87	Reduced fat content	62–71	98–99
Butter	81	99	Low fat content	41–61	98–99
Cheeses	12–28	20–68	Very low fat content	31–61	98–99
Egg yolk	33	66	Shortening	80–100	100
Egg white	0.02	0.15	Vegetable oil	100	100
Egg (whole, dried)	42	44	***Other foods***		
Foods of plant origin			Mayonnaise	80	95
Wheat flour (whole-grain)	2.5	2.8	Yeast	0,4	1.3
Wheat flour (white)	1.0–1.5	1.1–1.7	Mushrooms	0.4	3.3

In recent decades, the belief in the need to reduce fat intake in the diet has become widespread, which has also been reflected in the food products made available. Data on the fat content of meat in Table 3.23 relate to traditional products, but new breeds of pigs have very low fat content in muscle (only 2–3%). Also, new breeds of dairy cows have somewhat reduced fat content of milk (only 3.5–3.6%). Specially processed cheeses can have fat contents significantly lower than 20%. Palatability in such products is achieved by increasing their viscosity using soluble proteins or polysaccharides. The fat content in fish depends on their type and the time of year they are caught; for example, fatty fish include eel, carp and herring, while cod is an example of a lean fish. Emulsified fats often now have only 20–70% fat, margarine-type products and butter spreads tend to have only 30–40% or even lower amounts of fat. Reduced-fat mayonnaise and yoghurts are also available, along with many other reduced-fat products.

3.4.3.4.2 Composition

Previously, the belief was that fats were mixtures of simple triacylglycerols. However, it was then found that mixed triacylglycerols exist and that natural fats consist mainly of mixed triacylglycerols. It was also thought that fatty acids in the triacylglycerol molecules occurred randomly, but even this assumption was not correct. According to another theory, the composition of fatty acids depends on distribution of saturated (S) and unsaturated (U) fatty acids in triacylglycerols, therefore four possible types of triacylglycerols (G = glycerol) with random distributions of fatty acids are possible: SSS (GS3), SSU (GS2U), SUU (GSU2) and UUU (GU3). In triacylglycerols of a given type, saturated fatty acids (palmitic and stearic acids predominate) may substitute for each other. The same principle applies to unsaturated fatty acids (mainly oleic and linoleic acids). Because the experimental data did not correspond to this assumption, it was proposed that each fatty acid

tended to occur in as many molecules of triacylglycerols as possible. According to this theory, a fatty acid, which occurs in less than 33% of the total fatty acids, can be represented in each triacylglycerol molecule only once. If its ratio is between 33 and 67%, it can occur in the triacylglycerol molecule once or twice. Only if it is present in amounts higher than 67% can single triacylglycerols be formed in which this fatty acid occurs three times. From Figure 3.4, it is possible to deduct a percentage of each type of the triacylglycerols in which two fatty acids A and X occur in different ratios. Experimental data did support this theory for a long time, but it was necessary to revise this theory based on the assumption that the first compounds formed are specific 2-monoacylglycerols and then their positions C-1 and C-3 in the glycerol molecule are randomly occupied by the remaining fatty acids.

Today, these strict principles are not asserted because acyltransferases are not quite specific and deviations from this scheme occur often. There is a high variation in stereoisomers in oils and fats of different biological origin, typically with strong species specificity. Lard is unique among animal depot fats, because it has a strong predominance of palmitic acid in the *sn*-2 position (90–100%), most stearic acid is found in the *sn*-1 position, whereas position *sn*-3 is rich in oleic acid (Table 3.24). In beef tallow and other bovine adipose tissues, nearly 50% of the fatty acids in the *sn*-2 position are oleic acid and other acids, in decreasing order, are palmitic and stearic acids. Palmitic, stearic, and oleic acids are the major fatty acids in the *sn*-1 and *sn*-3 positions. In eggs, a high degree of asymmetry between positions *sn*-1 and *sn*-3 is found. Palmitic acid is mainly in the *sn*-1 position, whereas >60% of *sn*-2 fatty acids and *sn*-3 fatty acids is oleic acid. Linoleic acid predominates in the *sn*-2 position. Seed oils tend to have polyunsaturated fatty acids in the *sn*-2 position, but relatively little difference can be found between the positions *sn*-1 and *sn*-3, although less abundant fatty

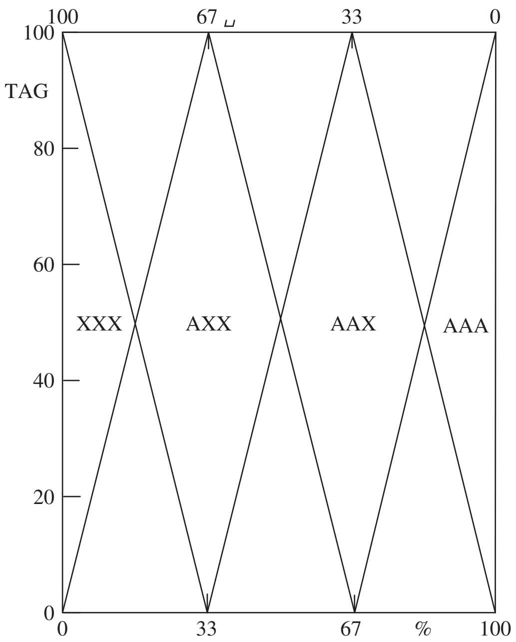


Figure 3.4 Distribution of fatty acids with acyls A and X in triacylglycerols of natural fats: TAG = % of triacylglycerols, % = % of fatty acid, XXX = TAG without acyl A, AXX = with one acyl A, AAX = with two acyls A, AAA = with three acyls A.

acids are often concentrated in the *sn*-3 position. Generally, saturated fatty acids (mainly palmitic acid) are in the *sn*-1 position and unsaturated fatty acids (mainly oleic acid) are in position *sn*-2. In triacylglycerols of marine mammals containing highly unsaturated fatty acids, such as DHA or EPA, prevail with combinations of two palmitic acids and DHA or EPA in the *sn*-1 position. In contrast,

Table 3.24 Typical composition of triacylglycerols of cocoa butter and pork lard.

Glycerol position[a]			Content (mol %)	
***sn*-1**	***sn*-2**	***sn*-3**	**Cocoa butter**	**Pork lard**
3 Saturated fatty acids				
P	P	P	0.3	0.3
P	P	S	1.7	0.9
S	P	S	2.4	0.6
P	S	P	0.2	0.2
P	S	S	0.1	0.4
S	S	S	0.2	0.3
1 Unsaturated fatty acids				
P	P	U	6.5	0.1
S	P	U	18.9	0.2
P	S	U	0.4	0.1
S	S	U	1.2	0.1
P	U	P	0.1	14.1
P	U	S	0.7	39.3
S	U	S	1.1	27.4
2 Unsaturated fatty acids				
U	P	U	36.5	0.0
U	S	U	2.4	0.0
P	U	U	2.9	6.4
S	U	U	8.3	8.9
3 Unsaturated fatty acids				
U	U	U	16.1	0.7

[a] P = saturated fatty acid C_{16} and lower, S = saturated fatty acid C_{18} and higher, U = unsaturated fatty acids.

binding of DHA or EPA is mainly at the *sn*-2 position in fish. The binding position tendency of DHA or EPA in triacylglycerol positional isomers consisting of two highly polyunsaturated fatty acids and one palmitic acid is the same as that for the combinations of one DHA (EPA) and two palmitic acids. The simplified composition of triacylglycerols of some other fats and oils is shown in Table 3.25.

The milk fat of mammals is very different from the fat depot, particularly in that it contains much higher amounts of triacylglycerols of fatty acids with shorter hydrocarbon chains and lower amounts of triacylglycerols of unsaturated fatty acids (Table 3.8). Characteristic triacylglycerols of milk fat have a number of carbons in fatty acids C_{44} (7.35%), C_{42} (7.62%), C_{34} (6.73%) and C_{32} (2.92% of total triacylglycerols), which can be used for detection of vegetable and animal fats in milk fat. Other triacylglycerols are: C_{54} (3.32%), C_{52} (7.76%), C_{50} (9.97%), C_{48} (9.09%), C_{46} (7.91%), C_{40} (9.70%), C_{38} (12.92%), C_{36} (12.12%), C_{30} (1.31%), C_{28} (0.66%), C_{26} (0.26%) and C_{24} (0,04). The last of these triacylglycerols can contain, for example, three bound caprylic acid or one butyric acid, one caprylic acid and one lauric acid, and so on.

The composition of triacylglycerols of plant seeds is usually very different from the composition of triacylglycerols in the pericarp, which is of practical importance for olive oil and palm oil. The difference is shown in Table 3.9. Also, the composition of the storage fat in animals differs (Table 3.10). Intermuscular fat of fish and chondrichthyans is fairly different from the liver oil (Table 3.12).

Table 3.25 Main groups of triacylglycerols in some fats and oils (in mol %).

Fat or oil	Type[a] SSS	SSU	SUU	UUU
Coconut[b]	82	18	0	0
Palm kernel[b]	61	38	1	0
Beef tallow	18	42	32	8
Pork lard	7	34	43	16
Cocoa butter	1	84	15	0
Palm	7	51	33	9
Maize germ	0	4	34	62
Cotton	<1	18	48	34
Peanut	0	9	44	47
Olive	0	5	35	60
Sunflower	0	3	26	71
Soybean	0	5	29	66
Linseed	0	5	43	52
Rapeseed 00	0	0	20	80

[a]S = Saturated fatty acid, U = unsaturated fatty acid.
[b]Fat contains mainly lauric and myristic acids.

Subcutaneous fat (the layer of adipose tissue) of mammals usually contains more triacylglycerols with unsaturated fatty acids than visceral fat (found around the viscera, especially the kidneys) that occurs in the abdominal cavity and is known as soft fat and leaf lard. However, these differences are not very significant.

3.4.3.5 Use

Most fats are used for nutritional purposes or as animal feed, either directly or after isolation from the food raw materials. Part of what is consumed, that added during cooking or frying, or that used as a spreadable fat is obvious fat, while part is concealed from the consumer, who is not directly aware that the food contains hidden fat. The ratio of both types of fats in human diet is approximately 1 : 1.

Oils and fats are also used for non-food purposes, such as the production of motor fuels, in oleochemistry and cosmetics. For these purposes, common edible oils and fats of lower quality are used. Some oils are obtained specifically for technological purposes, such as castor oil or tung oil. A typical example of the use of oils as fuel for diesel engines is the production of fatty acid methyl esters, especially from rapeseed oil. Oleochemicals from oils and fats manufacturing include fatty acids, fatty alcohols and other derivatives for the production of surfactants and subsequently detergents, paints, plastics, adhesives, building materials and many other products.

3.4.3.6 Procedures for obtaining fats and oils

The various methods for obtaining fats vary according to the raw material, and whether it is of animal or vegetable origin. Special procedures exist for bacterial fats that are designed for feed purposes. Owing to their high price, the use of bacterial fats is not very widespread. Metabolic engineering approaches are able to generate microbial strains with enhanced accumulation of triacylglycerols or free fatty acids that can be used as biodiesel precursors.

3.4.3.6.1 Procedures for vegetable fats and oils

On an industrial scale, the most commonly processed raw material is oil seeds. A summary of the important typical raw materials is given in Table 3.26. In terms of world oil production, the major oils are soybean, palm, rapeseed, sunflower, cotton, peanut, coconut, palm kernel, sesame and olive oils. A large number of other oils have only local significance, and a number of other oils are obtained solely for pharmaceutical, cosmetic and oleochemical purposes (linseed, castor and perilla oils in particular). Oils from the pericarp (pulp) of fruits, such as palm and olive oils, are obtained by specific procedures, unlike the oils from seeds.

The vegetable oils from seeds and beans are typically obtained by both physical extraction (mechanical expeller pressing) and chemical extraction, usually with hexane as a solvent (a petroleum-derived fraction without any aromatic hydrocarbons) and by a combination of both processes. If the seed oil content is lower than 25%, the oil is obtained by direct extraction. From seeds with higher

Table 3.26 Fat content in some raw materials.

Oil	Latin name of plant	Processed part of plant	Lipid content (%)
Coconut	*Cocos nucifera*	Seed (copra)	63–68
Palm	*Elaeis guineensis*	Pericarp	44–53
Palm kernel	*Elaeis guineensis*	Seed (kernel)	50–60
Olive	*Olea europea*	Pericarp	35–70
Olive kernel	*Olea europea*	Seed (kernel)	30–45
Almond	*Prunus amygdalus*	Seed	45–53
Hazelnut	*Corylus avellana*	Seed	50–65
Avocado	*Persea americana*	Seed	10–30
Sunflower	*Helianthus annuus*	Seed	22–36[a]
Peanut	*Arachis hypogaea*	Seed	45–55
Safflower (carthamus, kurdee)	*Carthamus tinctorius*	Seed	25–37
Sesame	*Sesamum indicum*	Seed	44–54
Cotton	*Gossypium hirsutum*	Seed (unpealed)	15–24[b]
Poppyseed	*Papaver somniferum*	Seed	36–50
Rapeseed (traditional)	*Brassica napus*[c]	Seed	38–45
Rapeseed (low erucic acid)	*Brassica napus*[d]	Seed	30–40
Mustard	*Leucosinapis alba*[e]	Seed	30–42
Soybean	*Soja max*	Seed	17–22
Linseed	*Linum usitatissimum*	Seed	35–45
Perilla	*Perilla frutescens*	Seed	42–51
Hempseed	*Cannabis sativa*	Seed	30–35
Maize	*Zea mays*	Germ	12–20
Wheat	*Triticum aestivum*	Germ	8–14
Rice	*Oryza sativa*	Bran	15–20

[a] Kernels contain up to 55% of oil.
[b] Kernels contain 30–38% of oil.
[c] Also known as turnip rape oil, colza oil, ravison oil, sarson oil: toria oil; produced from seeds of *Brassica napus*, *Brassica campestris*, *Brassica juncea* and *Brassica tournefortii* species.
[d] Also known as low erucic acid turnip rape oil, low erucic acid colza oil and canola oil; produced from low erucic acid oil-bearing seeds of varieties derived from the *Brassica napus*, *Brassica campestris* and *Brassica juncea* species.
[e] Derived from the seeds of white mustard (*Leucosinapis alba*, syn. *Sinapis alba* or *Brassica hirta*), brown and yellow mustard (*Brassica juncea*) and of black mustard (*Brassica nigra*).

oil content, most of the oil is obtained by pre-pressing thus reducing the fat content to 17–20% in the meal. Pre-treatment is necessary to produce leakage of the oil from the seeds, which consists of milling followed by exposure to water at elevated temperatures. This causes tissue disruption and then the subsequent disruption of cell walls and membranes. The remaining oil in the meal is usually obtained by extraction using a continuously operating extractor, which lowers the residual fat content in meal to 1.5–2%. The solution obtained by extraction contains 30–40% of the oil in hexane, the so-called **miscela**, from which the solvent is evaporated in an evaporator under reduced pressure, and any residual solvent is then removed by steam distillation under reduced pressure. The so-called crude oils (obtained by physical extraction and chemical extraction) have higher acid contents and their organoleptic characteristics render them unsuitable for consumption in the natural state. Thus these oils have to be refined.

The so-called **virgin oils**, suitable for direct consumption, are obtained by grinding the material and extracting the oil by mechanical means (pressing). The most important virgin oils are oils from olives, but other virgin oils are now also appearing on the market.

Virgin olive oil is the oil obtained from the fruit of the olive tree (*Olea europea*, Oleaceae) by mechanical or other physical means, under conditions (particularly thermal) which do not lead to alteration of the oil (see Section 3.4.3.7).

In addition to homolipids, crude vegetable oils contain heterolipids and other components of plant material. The heterolipids content depends on the technology used. The oil obtained through pressing contains 0.1%, while the extracted oil contains 1–2% and soybean oil contains 3–4% phospholipids. The heterolipids content of virgin oils is much lower, about 0.01%.

3.4.3.6.2 Procedures for animal fats

Animal fats are mostly obtained by the action of hot water, when the fat is washed from the tissue and then separated from the aqueous phase. Previously animal fats were obtained using hot steam and by direct heating (rendering), as is still practiced in the home. Rendered fats have a distinctive flavour due to the pyrolytic products of proteins contained in adipose tissue, while the unrendered fat obtained using hot water is virtually odourless and tasteless.

Milk fat is usually obtained from milk in the form of butter. The milk fraction rich in fat (cream) is separated from the aqueous phase poor in fat (skimmed milk) by centrifugation. The cream is an oil-in-water emulsion (in short o/w emulsion) from which the fat (butter) is mechanically separated by inversion to a water-in-oil emulsion (w/o), in which milk proteins act as emulsifiers. The residual liquid, which remains is buttermilk. Butter contains at least 80% fat, about a 2% non-fat portion (proteins, carbohydrates and other substances) and the rest is water. It generally has a pale yellow colour, which is dependent on the animals' feed. Some food colourings, most commonly annatto or β-carotene, are used in the commercial manufacturing process. Butter is most frequently made from cows' milk, but can also be manufactured from the milk of other mammals, including sheep, goats, buffalo and yaks. For specific applications, anhydrous milk fat can be obtained. In India, Pakistan and the Middle East anhydrous milk fat, known as ghee, is prepared by heating butter to evaporate the water and separate the precipitated proteins. Butter for particular applications can also be processed by fractional crystallisation.

3.4.3.7 Vegetable oil refining

Unlike animal fats, which are usually used without further treatment, vegetable oils obtained from seeds by mechanical expelling and solvent extraction have unpleasant organoleptic properties. Therefore, these oils are refined in order to be more acceptable to consumers. The refining process includes:

- **degumming** (hydration)
- **deacidification** (neutralisation)
- **bleaching**
- **deodorisation** or **physical refining**.

The crude oil is first centrifuged or filtered to remove solid particles (impurities, seeds or parts of the cellular tissue), which are the main causes of undesirable enzyme activities. The next step is hydration, which separates out a substantial portion of the heterolipids (mainly phospholipids) as hydrated gums that are highly insoluble in oil and can be quickly separated out as a hydration sludge. Plant mucilages (see Section 4.5.1.8), carbohydrates and proteins are also separated. Hydration is based on heating crude oil with water or, in modern processes such as superdegumming, total degumming and so on, with concentrated hydrochloric, phosphoric or citric acids and subsequent separation of the hydration sludge. During the process of hydration with water, the hydration sludge is evaporated and used in the production of lecithin.

In the next step, fatty acids are separated from the oil by neutralisation (deacidification or alkaline refining) with sodium hydroxide solution (which is also effective in the removal of toxic gossypol from cottonseed oil; see Section 9.11). The free fatty acid content varies widely within 0.5–1.5% in seed oils from raw materials grown in a temperate zone. After neutralisation, the fatty acid content falls below 0.1%. The resulting soaps are separated as soap stock and typically used in animal feed. Tropical oils, such as palm and coconut oils, contain 3–7% free fatty acids. It is therefore preferable to separate the free fatty acids together with volatiles by distillation in the end stage of physical refining process. In this case, the main components of the distillate are free fatty acids.

Bleaching is achieved by adsorption on activated bleaching clays (sorbents of the aluminium silicate type) or in combination with other adsorbents (such as activated carbon, also known as activated charcoal) to remove oil soluble pigments (such as carotenoids and chlorophylls), residual phospholipids, and eventually soap residues resulting from the deacidification process. Volatile substances are removed by deodorisation via steam distillation under reduced pressure. The volatile compounds are mainly responsible for the unpleasant smell and aftertaste of crude oil, so this process provides organoleptically neutral, indifferent oils.

Because the deodorisation process conditions (as well as those for physical refining) are adjusted for free fatty acids distillation, during deodorisation a partial loss of tocopherols and sterols occurs. They pass into the distillate, or deodorisation condensate, from which it is possible to recover them subsequently. An undesirable phenomenon in deodorising (physical refining), in addition to partial loss of sterols and tocopherols, is partial geometrical isomerisation of double bonds of polyenoic fatty acids. By adjusting the process parameters, these adverse effects can be minimised. The refining process simultaneously leads to decomposition of hydroperoxides, and other oxidation products of fatty acids and volatile secondary oxidation products are distilled off. The vitamin and carotenoid pigments content of the lipophilics is partly reduced, but the nutritional value of the oil is not altered significantly. The resulting refined oil is virtually a pure mixture of triacylglycerols with small amounts of partial esters of glycerol, residual free fatty acids and some desirable accompanying substances (in particular phytosterols, tocopherols, fat-soluble vitamins and carotenoids).

Winterisation is the process of removing components with high melting point (such as waxes) from some vegetable oils, for example sunflower, rice bran and cotton seed oils or partially hydrogenated

soya bean oil. For vegetable oils this is necessary to ensure that the oil remains a clear liquid even when stored at low temperatures for extended periods of time. The bleached oil is cooled down to the winterisation temperature and after maintaining this for a certain length of time the wax crystals are separated from the oil by means of pressure filtration or centrifugal separators.

According to the International Olive Oil Council (IOOC), there are four commercial types of olive oils: extra virgin olive oil, virgin olive oil, olive oil and pomace oil. Virgin oils are obtained entirely through physical methods (pressing and heating), extra virgin olive oil contains up to 0.8% of free fatty acids (expressed as oleic acid) and virgin olive oil contains up to 2.0% of this acid. Virgin olive oils are only filtered and not refined. Olive oil is a mixture of refined oil (oils of lower quality are refined by filtration through activated carbon) with virgin oil. Pomace oil (obtained from pomace, which is the ground pulp and seeds, by solvent extraction and other physical methods and refining of the oil thus obtained) is also a blend of refined and virgin olive oil. The oleic acid content of the last two types is up to 1.0%.

3.4.3.8 Crystallisation and emulsification of fats

Vegetable oils are often eaten directly (100% cooking fats or oils). The basic problem is that most vegetable oils are liquid, while what are usually required are solid fats of suitable consistency, which have similar properties to traditionally used animal fats, such as butter and lard. Therefore, vegetable oils are processed further to fats of suitable consistency. Vegetable oil used for the production of margarines and other water-in-oil emulsified spreads and shortenings for use as dough fat or filling fat is called **fat blend**. Fat blend is made by mixing the so-called **structural fat** and liquid oil. Structural fat contains a mixture of triacylglycerols of higher melting points, which crystallise in the fat blend and create a crystalline structure. The fat blend for the manufacture of shortenings requires the formation of a dispersion of crystals in oil (β-crystalline modification; see Section 3.4.3.9), while fat blend for the manufacture of margarines requires the formation of a spatial network (β′-crystalline modification). Triacylglycerol crystals produce the so-called solid fat content (SFC), which is defined as the percentage content of the solid phase at a constant temperature (usually in the temperature range of 10–40 °C). Liquid triacylglycerols (the fat liquid content) are adsorbed (immobilised) on the surface of the crystals or in the spatial network of crystals. Structural fat carries the fat blend structure and its physical and textural characteristics. Structural fat can be produced by:

- **partial catalytic hydrogenation**
- **ester interchange (transesterification)**
- **fractionation**.

Structural fat is currently no longer produced by partial catalytic hydrogenation, because the presence of *trans*-isomers in fats is considered undesirable from a nutritional point of view. Fractionation of triacylglycerols (fractional crystallisation) is also marginally employed. The main process of structural fats production is transesterification of triacylglycerols.

Shortenings are made of fat blends, which crystallise in a controlled manner, forming a dispersion of crystals of structural triacylglycerols (ideally, crystals of triacylglycerols form a special network) in which the dispersion medium are liquid triacylglycerols. Shortenings are used for frying and as fats that are added to most doughs and batters to increase the plasticity and workability of doughs, and to impart a crisp and crumbly texture to baked products. In terms of consistency, shortenings can be liquid, semiliquid or hard. Whipped fats are solid foams with inert gas (nitrogen) as a dispersed (internal) phase at a level of 15–20% by volume, and are suitable for frying.

A classic emulsified fat is **margarine**, which contains at least 80% of fat and the rest is the aqueous phase (minimum 16% of total emulsion content by weight). It is obtained by mechanically emulsifying fat blends, containing an emulsifier (usually monoacylglycerols with the addition of diacylglycerols, often mixed with lecithin), with an aqueous phase and by subsequent crystallisation. Margarine and butter both consist of a water-in-oil emulsion (w/o). Emulsification occurs in parallel with the gradual crystallisation of structural lipids (triacylglycerols of higher melting points). Emulsified oil is a three-phase colloidal system consisting of a dispersion medium of liquid triacylglycerols, droplets of water (10–80 μm in diameter) that represents the dispersed phase forming the emulsion and a dispersion of triacylglycerol crystals in a stable crystalline form. Ideally, triacylglycerol crystals create a spatial network and form a gel. Margarines are produced either as spreadable fats or in a harder consistency for baking purposes. Today, most emulsified fats are produced with a lower lipid phase content (70, 60 or only 40% of fat, or only 20–25% of fat), which are used as fat spreads.

Another type of emulsified fat is **mayonnaise**, which is flavoured with oil and water emulsions, where the emulsifier is egg yolk (or strictly the phospholipids present in it). Today, many similar products with lower oil contents are produced in abundance. Thus, mayonnaise consists of an oil-in-water emulsion (o/w). A similar composition is found in tartar sauce and some salad dressings, but the fat content is usually lower in these products. Non-food fat emulsions are used for paints, cosmetics, pharmaceuticals and other purposes.

3.4.3.9 Composition, crystallisation and properties of fats and oils

Food products referred to as fats and oils are not just a mixture of triacylglycerols with small amounts of monoacylglycerols and diacylglycerols. They also contain up to 1–2% of accompanying substances (sometimes more), which are mainly sterols and other terpenoids. Small amounts of lipophilic vitamins, hydrocarbons, phospholipids and traces of other compounds are also present. Fats and oils are insoluble in water and very slightly soluble in alcohol. Their properties are mainly determined by their fatty acid composition.

Fats can be hard and brittle substances (cocoa butter), hard substance (beef tallow), gooey solids (pork lard, palm oil) and viscous liquids (vegetable oils). The melting and solidification points of some fats are listed in Table 3.27. The range of values is mainly related to the variability of the composition of fatty acids and triacylglycerols.

Solid fats, as well as fatty acids, are polymorphic substances. Although the triacylglycerols form the main crystalline phase, the minor components can often play a significant role in how crystallisation occurs and this may be substantially different in refined oils to that in the unrefined starting material. On slow cooling from the liquid, the lipid molecules have time to organise and can eventually form coherent, three-dimensional crystals. The arrangement of the molecules into the crystalline state depends on such factors as the cooling rate, the temperature at which crystallisation occurs, the agitation rate and the composition of the lipid phase. Crystallisation of pure triacylglycerols can lead first to less stable γ- and α-polymorphic forms of low density, from which the metastable β′-polymorphic forms arise that are then transformed into the more stable β-forms of highest density with the highest melting points (Table 3.28). For example, on cooling, liquid tristearin generates first the α-form, which is, on further cooling, transformed into the β-form. Cooling the melt a few degrees centigrade higher than the temperature that corresponds to the melting point of the α-form creates the β′-form, heating of the β′-form to a higher temperature than the melting point creates the β-form. The orderliness of the molecules in the crystal lattice increases with increasing density, but the entropy and Gibbs energy decrease and reach minimum values in the stable modification.

The individual modifications differ in the arrangement of triacylglycerol molecules in the crystal lattice. The α-modifications of triacylglycerols form crystals in a hexagonal, β′-modifications in an orthorhombic and β-modifications in a triclinic crystal lattice. In the crystalline lattice, triacylglycerol molecules are oriented in a chair or tuning fork configuration. Crystals of triacylglycerols in the α-modification containing residues of the same fatty acids tend to form tuning fork configurations with the fatty acid chains perpendicular to the crystal planes. Crystals in the β′-modification have tuning fork configuration with angled fatty acids chains. Chains of fatty acids in positions C-1 and C-3 of glycerol are opposite to the acid chain in position C-2 and inclined to the crystal planes. Crystals of triacylglycerols in the β-modification have chair configuration (Figure 3.5).

Natural triglycerides contain different fatty acids in the individual positions of the glycerol, therefore there are numerous deviations from the above described behaviour during crystallisation. For example, one of the bound fatty acids can differ significantly from the other two fatty acids in its chain length (by four or more carbon atoms), or in the case of unsaturated fatty acid, the layer of fatty acids does not have the depth of two (type β-2), but of three chains (type β-3) of fatty acids (Figure 3.6). Usually there are several polymorphic forms, one of which predominates.

Table 3.27 Melting and solidification points of solid fats.

Fat	Melting point (°C)	Solidification point (°C)	Fat	Melting point (°C)	Solidification point (°C)
Pork lard	28–40	22–32	Coconut oil	20–28	18–23
Beef tallow	40–50	30–38	Palm oil	30–37	27–43
Cows' milk fat	28–38	15–25	Cocoa butter	32–36	21–27
Breast milk fat	30–32	22–23	Hydrogenated vegetable oils	28–42	23–36

Table 3.28 Polymorphic forms of triacylglycerols.

	Melting point of polymorphic forms (°C)					Melting point of polymorphic forms (°C)			
Triacylglycerol	γ	α	β′	β	Triacylglycerol	γ	α	β′	β
Tricaprylin	-	-	−21.0	8.3	Trilinolein	-	−45.0	−13.0	−10.0
Tricaprin	-	−15.0	18.0	31.5	Trilinolenin	-	−44.6	-	−24.0
Trilaurin	-	15.0	35.0	46.5	1,2-Dipalmitoyl-3-oleoylglycerol	18.5	28.9	-	34.8
Trimyristin	-	33.0	46.5	57.0	1,3-Dipalmitoyl-2-oleoylglycerol	18.0	26.5	-	38.5
Tripalmitin	-	45.0	56.5	65.5	1,2-Dipalmitoyl-3-linoleylglycerol	-		-	26.5
Tristearin	-	55.0	65.0	72.5	1,2-Distearoyl-3-oleoylglycerol	30.4	43.5	-	-
Triolein	-	−32.0	−12.0	5.5	1,3-Distearoyl-2-oleoylglycerol	22.4	37.0	41.5	44.3

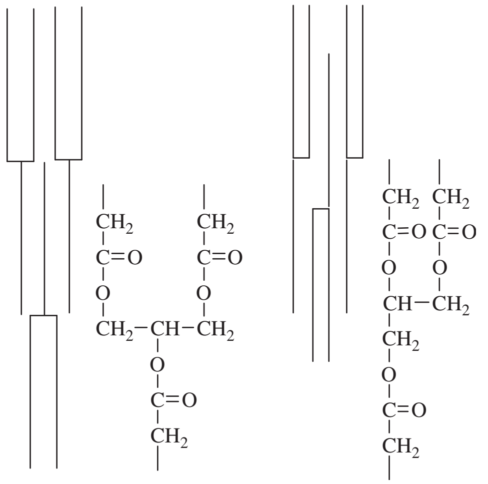


Figure 3.5 Tuning fork (β′-modification) and chair configuration (β-modification) of triacylglycerols in crystal lattices.

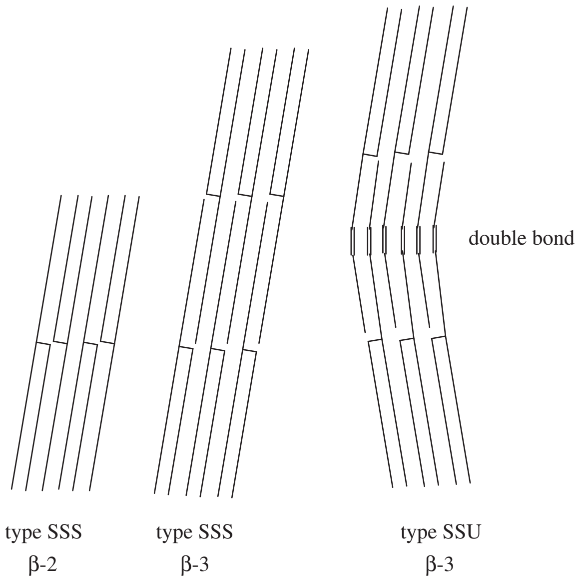


Figure 3.6 Arrangement of polymorphic forms of triacylglycerols in crystals.

Heterogeneous fats containing different fatty acids of different chain lengths and fats with asymmetric distribution of fatty acids (SSU or UUS types) tend to occur in the metastabile β′-modification, which is slowly transformed into the β-modification. Homogeneous fats containing fatty acids of roughly the same length and fats with a symmetrical distribution of fatty acids (SUS type) are very quickly transformed into the stable β-modification.

Coconut and palm kernel oil, palm oil, traditional rapeseed oil and cottonseed oil, beef tallow and butter tend to preferentially crystallise in the metastabile β′-modification, which has characteristic small needle-like crystals. These triacylglycerols characteristically form clusters and aggregates to form the crystal spatial network, ideally they form a gel structure, which shows thixotropy. Fats crystallising in this modification are suitable for the production of margarines and shortenings. At a suitable temperature, when a part of the crystals occur in the matrix formed by the liquid fat, these fats impart the desired consistency to products. In dough and pastry, these fats help incorporate air bubbles and suspended particles of flour and sugar.

Olive, peanut, sunflower, soybean, low erucic acid rapeseed and safflower oils, cocoa butter and pork lard crystallise in the stable β-modification, for which large fat crystals conferring grainy texture to the fats are characteristic. Such crystals arise, for example, in pork lard during storage in a refrigerator. Association of crystals in the β-modification is significantly less than in the β′-modification, and therefore fats in this configuration of the crystal lattice do not create a crystalline network, but a simple dispersion of fat crystals in liquid triacylglycerols, which is a sol (see Section 7.8.3.1).

In many food products, it is important to control lipid crystallisation in order to obtain the desired number, size distribution, polymorph and dispersion of the crystalline phase. Proper control of the crystalline microstructure leads to products with the desired textural properties and physical characteristics. For example, tempering of chocolate prior to molding or enrobing is designed to controlled crystallisation of the cocoa butter into a large number of very small crystals that are all in the desired polymorphic form. When controlled properly, the cocoa butter crystals in chocolate contribute to the desired appearance (the shine or gloss), meltdown rate upon consumption and stability during shelf life (prevention of fat bloom, the gray film of fat on the surface). Natural cocoa butter has a total of six polymorphic forms (γ, α, β'_2, β'_1, β_2 and β), the melting points of which range from 17.3 to 36.3 °C. Only the fifth form (β_2) with a layer corresponding to three lengths of the fatty acid chains (the main triacylglycerols are StOSt, POSt and POP, Table 3.24) and melting at 33.8 °C has the desirable organoleptic properties. To ensure that this form is created in the production of chocolate requires tempering. Liquid chocolate is allowed to cool during tempering. When the fat begins to crystallise, it is heated to a temperature just below the melting point of the desired polymorphic form when the undesired polymorphic forms melt. Chocolate is stirred at this temperature for some time to ensure that a greater proportion of fat is crystallised into small fine crystals. Inappropriately tempered chocolate, or chocolate exposed to fluctuations in temperature, creates a fat bloom as a result of the fat transformation into more stable polymorphic forms, which then crystallise on the chocolate surface.

3.4.4 Esters of polyhydric alcohols

This group includes industrially produced substances. Esters of sorbitol or saccharose with one or two molecules of fatty acids are used as emulsifiers of fats. Sucrose esters with 5–8 molecules of fatty acids are used as fat substitutes (called Olestra), with the advantage that they have physical properties similar to fat and give the same sensory impression, but they are unusable in the human body and therefore do not supply the body with energy. These substances are described in more detail in the section dealing with food additives (see Section 11.5.2.1.5).

3.5 Heterolipids

Unlike homolipids, heterolipids contain not only bound fatty acids and alcohols, but also other components, and, depending on these, they are further subdivided into:

- **phospholipids**
- **glycolipids**
- **sulfolipids**.

The most important group of phospholipids are **glycerophospholipids**. Homolipids derived from glycerol (such as mono-, di- and triacylglycerols), glycerophospholipids and **glyceroglycolipids** together constitute one class of lipids known as **glycerolipids**. Another major group of lipids are **sphingolipids**, which are not derived from glycerol and are not very important in nutrition. Sphingolipids include fatty acid amides derived from nitrogen-containing alcohols known as **sphingosines**. Sphingolipids are further divided into **sphingohomolipids** (***N*-acylsphingosines** called **ceramides**) and **sphingoheterolipids** (that include **sphingophospholipids** and **sphingoglycolipids**). Glycerophospholipids (formerly known as phosphoglycerides), glyceroglycolipids and sphingolipids are structural lipids of biological membranes.

3.5.1 Phospholipids

3.5.1.1 Structure and nomenclature

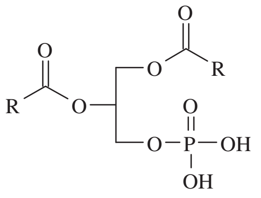
The most important group of phospholipids are glycerophospholipids, which are glycerol derivatives esterified with both fatty acids and phosphoric acid. Glycerophospholipids are divided into three groups according to the number of bound fatty acids, other types of bonds and the structure of the bound components:

- **phosphatides**
- **lysophosphatides**
- **plasmalogenes**.

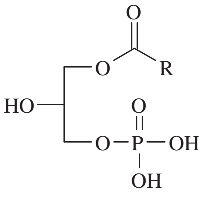
3.5.1.1.1 Phosphatides and lysophosphatides

Phosphatides are derivatives of phosphatidyl residues, which are based on 1,2-diacyl-*sn*-glycerol, which contains bound phosphoric acid on the C-3 hydroxyl. The basic compound is 3-*sn*-phosphatidic acid (1,2-diacyl-*sn*-glycerol 3-phosphoric acid) (**3-53**). If only position C-1 (which is more often than position C-2) is occupied by an acyl, the resulting residue derived from 1-acyl-*sn*-glycerol is called lysophosphatidyl. The corresponding acid is 3-*sn*-lysophosphatidic acid (1-acyl-*sn*-glycerol 3-phosphoric acid) (**3-54**).

In phosphatides and lysophosphatides, phosphoric acid is further esterified by some hydroxy compounds, namely by choline (*N*,*N*,*N*-trimethylethanolamine), ethanolamine (2-aminoethanol), L-serine, *myo*-inositol and glycerol.

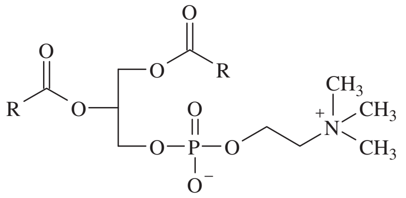


3-53, phosphatidic acid

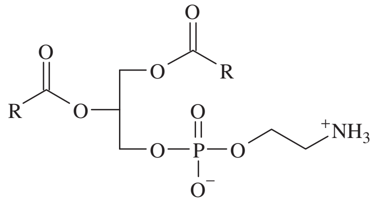


3-54, lysophosphatidic acid

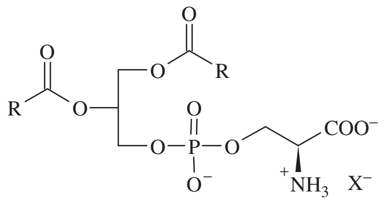
The most frequently occurring phosphatide is the ester with amino alcohol choline, which is known as (3-*sn*-phosphatidyl)choline or 1,2-diacyl-*sn*-glycero-3-phosphocholine, or phosphatidylcholine for short (**3-55**). Previously, this ester was known as lecithin, but this name should no longer be used for the pure compound, but only for the industrial concentrate of phospholipids. If ethanolamine (colamine) is bound to the phosphatidyl residue, the resulting phospholipid is called (3-*sn*-phosphatidyl)ethanolamine or 1,2-diacyl-*sn*-glycero-3-phosphoethanolamine (phosphatidylethanolamine; **3-56**). Previously, the fraction containing phosphatidylethanolamine, together with other phospholipids, was known as cephalin. This name has been used only rarely in non-chemical literature, and should not be used because it is a poorly defined mixture of compounds. Another phospholipid is (3-*sn*-phosphatidyl)-L-serine or 1,2-diacyl-*sn*-glycero-3-phospho-L-serine (phosphatidylserine, **3-57**). In the toxic skin secretion of fish of the Ostraciidae family



3-55, phosphatidylcholine



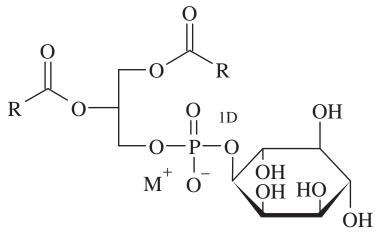
3-56, phosphatidylethanolamine



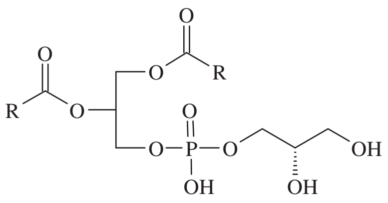
3-57, phosphatidylserine (X$^-$ = counter anion)

choline is bound as esters of 3-acylhexadecanoic acids (see Section 10.3.2.11.2).

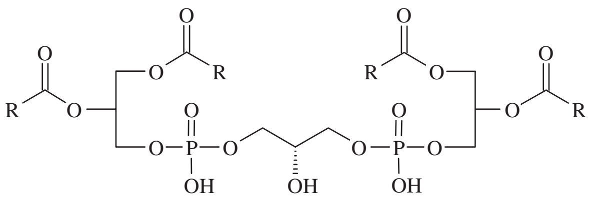
The compound of this group that does not contain nitrogen is (3-*sn*-phosphatidyl)-*myo*-inositol or 1,2-diacyl-*sn*-glycero-3-phospho-*myo*-inositol (or phosphatidyl-*myo*-inositol for short, **3-58**), which may have the second phosphoric acid bound to *myo*-inositol via the C-4 hydroxyl group (see Section 4.3.1.1.2). Another compound of this group that does not contain nitrogen is (3-*sn*-phosphatidyl)-*sn*-glycerol or 1,2-diacyl-*sn*-glycero-3-phospho-*sn*-glycerol (phosphatidylglycerol, **3-59**). Phosphatidylglycerol is found as a minor component in various animal and plant tissues, particularly in chloroplasts. The structurally related compound is cardiolipin (**3-60**), an acyl derivative of 1′,3′-di-*O*-(3-*sn*-phosphatidyl)-*sn*-glycerol, which was first isolated from cardiac muscle, hence its name.



3-58, phosphatidyl-*myo*-inositol (M^+ = counter cation)



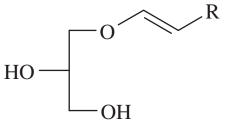
3-59, phosphatidylglycerol



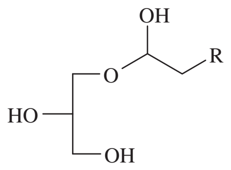
3-60, cardiolipin

3.5.1.1.2 Plasmalogens

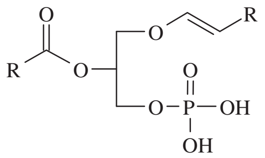
The acyl in the *sn*-1 position of glycerol in plasmalogenes (phosphate esters of glycerylethers) is replaced by a higher aliphatic aldehyde bound as a hemiacetal. It usually occurs in a dehydrated form (**3-61**), but may be also present as a hydrate (**3-62**). If the *sn*-3 position of glycerol is esterified with phosphoric acid then this plasmalogen is formally derived from 3-*sn*-plasmenic acid, the systematic name for which is 2-acyl-1-(alk-1′-en-1′-yl)-3-*sn*-glycerol 3-phosphate (**3-63**). Depending on the nitrogenous bases bound in plasmalogenes, 2-acyl-1-(alk-1′-en-1′-yl)-3-*sn*-phosphocholine (for short plasmenylcholine), 2-acyl-1-(alk-1-en-1-yl)-3-*sn*-phosphoethanolamine (plasmenylethanolamine) and 2-acyl-1-(alk-1-en-1-yl)-3-*sn*-phospho-L-serine (plasmenylserine) are recognised. The corresponding compounds with a saturated chain in the *sn*-1 position also exist. They are derived from 3-*sn*-plasmanic acid, that is, 2-acyl-1-alkyl-*sn*-glycerol 3-phosphate (**3-64**). Such a compound is, for example, 2-acyl-1-alkyl-3-*sn*-phosphocholine (briefly plasmanylcholine). Plasmalogenes probably show cytostatic effects based on the inability of tumour cells to metabolise their molecules.



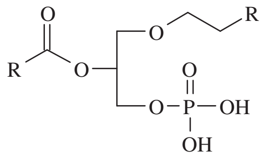
3-61, glyceryl ether (dehydrated form)



3-62, glyceryl ether (hydrated form)



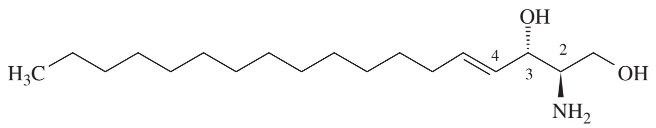
3-63, plasmenic acid



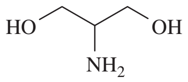
3-64, plasmanic acid

3.5.1.1.3 Sphingophospholipids

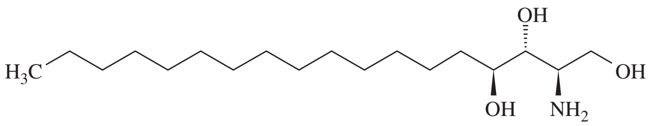
An important group of phospholipids, the sphingophospholipids, do not contain glycerol, but the *N*-analogues of glycerol generally known as sphingosines. Sphingosines (2-amino-1,3-dihydroxyalkanes), are bases with a long chain substituted on the nitrogen atom by acyls of fatty acids with 14–26 carbon atoms. In mammals, this base is usually (*E*)-sphing-4-enine, (2*S*,3*R*,4*E*)-2-aminooctadec-4-ene-1,3-diol also known as (*E*)-D-*erythro*-2-amino-1,3-dihydroxyoctadec-2-ene or sphingosine (**3-65**). Plants contain up to eight sphingoid-C_{18} bases derived from D-*erythro*-sphinganine, also known as (2*S*,3*R*)-sphinganine. As a result of *cis*- or *trans*-dehydrogenation at C-8, the prevalent regioisomers are (*E*/*Z*)-sphing-8-enine (C18:1), (4*E*/8*E*/*Z*)-sphingadi-4,8-enine (18:2) and (8*E*/*Z*)-4-hydroxysphing-8-enine. In bacterial lipids, a common component is 2-aminopropane-1,3-diol derived from L-serine, which is called serinol (**3-66**). In the yeast *Saccharomyces cerevisiae* 3-hydroxysphinganine predominates, which is known by its systematic name of (2*S*,3*S*,4*R*)-2-aminooctadecan-1,3,4-triol, or also phytosphinganine, C18:0 (**3-67**).



3-65, sphingosine

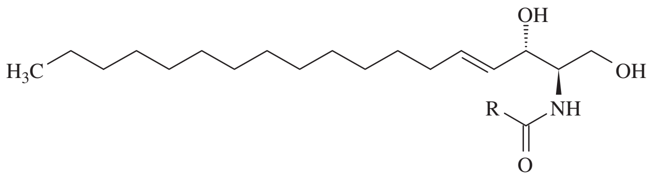


3-66, serinol

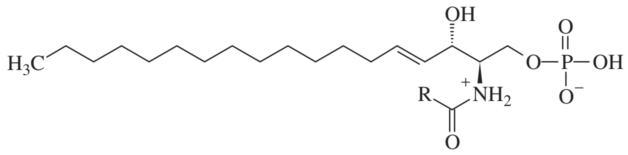


3-67, phytosphinganine

The amino group of sphingosine linked to a fatty acid via an amide bond occurs in the *N*-acylsphingosines known as ceramides (**3-68**), which belong to the homolipids. If the primary hydroxyl group of ceramide (sphingosine) is esterified with phosphoric acid, the resulting phospholipid is called ceramide phosphate (**3-69**). Ceramide phosphate esterified with choline is known as sphingomyelin (or ceramide phosphorylcholine) (**3-70**). Sphingomyelin is thus the sphingolipid analogue of phosphatidylcholine.



3-68, ceramide



3-69, ceramide phosphate

The composition of fatty acids linked to phospholipids differs from the fatty acid composition of lipid reserves of the

Table 3.29 Fatty acid composition of phospholipids of different origin (% of total fatty acids).

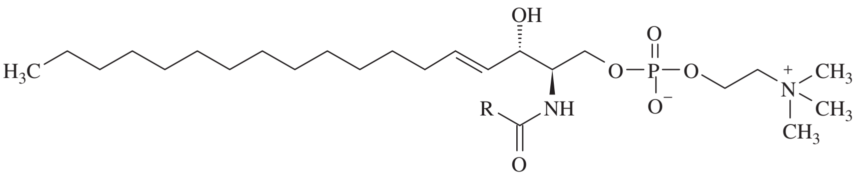
	Phospholipid				
Fatty acid	Soybean	Sunflower	Rapeseed	Egg	Milk
Palmitoic	14	15	12	32	20
Stearic	3	6	5	4	16
Oleic	14	19	18	46	60
Linoleic	58	46	52	4	2
Linolenic	4	0	1	0	0

same organism. Phospholipids typically contain higher amounts of palmitic acid, usually bound in the *sn*-1 position, and linoleic acid, which is most often bound in the *sn*-2 position. Some phospholipids contain large amounts of higher aliphatic hydroxy acids. The fatty acid composition of phospholipids of different origins is given in Table 3.29. The composition of fatty acids in individual soybean phospholipids is shown in Table 3.30. Phospholipids, particularly phospholipids of seafood, contain small amounts of arsenic analogues (see Section 6.2.3.1).

3.5.1.2 Biochemistry, physiology and nutrition

Phospholipids are essential components of animal and vegetable organisms, in which they are present as a part of the cellular and intracellular membrane and as components of lipoproteins that are stabilised due to the emulsifying ability of phospholipids. The phospholipids content also affects the solubility of low density lipoproteins (see Section 3.2.1) in blood plasma.

Phospholipids are formed from precursors by the action of several phospholipases (Table 3.31), which catalyse the connection of individual components to glycerol. The same enzymes catalyse the cleavage of phospholipids. Phospholipids are not an essential food for humans because the body can synthesise the basic building blocks through at least two different mechanisms. The first uses the appropriate alcohol (such as choline and ethanolamine) activated by binding to CDP (cytidine 5′-monophosphate), which is then connected to phosphatidic acid. Another possibility is the reaction of an alcohol with 1,2-diacylglycerols (activated by binding to CDP). In the absence of the basic building units, a further, third, possibility that can take place in the body is the direct synthesis of phospholipids. Decarboxylation of glycerophosphatidylserine



3-70, sphingomyelin

Table 3.30 Composition of fatty acids bound in soy phospholipids (% total fatty acids).

Type of phospholipid	Fatty acid				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Phosphatidylcholine	14–20	4–6	8–14	58–65	2–6
Position *sn*-1	34	8	19	33	4
Position *sn*-2	1	1	10	78	10
Phosphatidylethanolamine	15–30	3–5	5–9	53–65	3–6
Phosphatidic acid	34	8	12	45	1
Phosphatidylinositol	25–48	8–12	8–9	36–45	2–7

Table 3.31 Phospholipases.

Phospholipase	Activity	Origin and characteristics
A	A1: splits fatty acids bound in the *sn*-1 position; A2: splits fatty acids bound in the *sn*-2 position	Pancreas, intestinal mucosa, microorganisms (e.g. *Escherichia coli* and *Saccharomyces cerevisiae*)
B	Splits acyl in the *sn*-2 position, then acyl in the *sn*-1 position	Microorganisms (e.g. *Penicillium notatum*)
C	Splits bonds between the residue of glyceroland bound phosphotic acid	Microorganisms (e.g. *Bacillus cereus*, *Clostridium perfringens* and *Clostridium welchii*)
D	Splits bonds between phosphoric acidand nitrogen base	Vegetables, nuts, legumes

yields glycerophosphatidylethanolamine and, subsequently, glycerophosphatidylcholine is produced by methylation of the amino group of the ethanolamine residue. Of course, it is advantageous if the diet contains adequate amounts of phospholipids, because the body has sufficient building material for their re-synthesis. For this reason, various phospholipid preparations are currently recommended for dietetic purposes.

3.5.1.3 Occurrence

Phospholipids are found in all plants and animals as a part of the cellular and intracellular membranes. Their content is about 1% of dry matter (Table 3.32) and is not increased even in depot fat. Some animal tissues are particularly rich in phospholipids, especially the nervous tissue and egg yolk. Egg yolk contains about 28% of phospholipids and constitutes an appropriate source of phospholipids for pharmaceutical purposes. Soybeans are also a relatively rich source of phospholipids, but their phospholipid composition is very different to that in eggs (Table 3.33). Soy and egg phospholipids are used as emulsifiers.

When obtaining oil from oilseeds, a considerable amount of the phospholipids are extracted into the lipid phase, from which it is possible to isolate the phospholipid fraction by adding water or acidic solutions, such as phosphoric or citric acids that release phospholipids from their salts. The phospholipids are hydrated, become less lipophilic, and therefore are excluded from the lipid phase. The obtained product is called **lecithin**.

3.5.1.4 Lecithin

As already mentioned, the process of degumming converts the majority of phospholipids into hydrated gums that are highly insoluble in oil and are quickly separated as a hydration sludge. Evaporation of water under reduced pressure gives a dark brown phospholipid concentrate, which is called lecithin. Lecithin contains 30–50% of homolipids (acylglycerols), free fatty acids, sterols and tocopherols and various other substances (e.g. sugars, which represent about 6.5% of soy lecithin, amino acids and metal ions) as well as chlorophylls and carotenoid pigments. In addition to glycerophospholipids, lecithin contains other heterolipids of which the major group is glyceroglycolipids (6–7% of soy lecithin).

Food lecithin is made from specially selected high quality crude oils, because its refining is difficult and bleaching using hydrogen peroxide is not permitted in Europe. For some purposes, lecithin is subjected to fractionation to increase the phosphatidylcholine content, which is a more valued compound than other phospholipids.

Table 3.32 Content of phospholipids in foods.

Food	Content (% dry matter)	Food	Content (% dry matter)
Animal materials		Pork lard	0.01–0.1
Brain	5–6	Butter	0.6–1.4
Liver	3–4	***Plant materials***	
Heart	2.6–3.0	Vegetable oils	0.02 (refined)
Kidney	1.6–3.0		0.6–3.2 (raw)
Egg yolk	28	Fruits, vegatables, cereals	0.5–1.5

Table 3.33 Distribution of individual phospholipids in the phospholipid fraction (% of total phospholipids).

	Phospholipid				
Compound	**Rapeseed**	**Soybean**	**Milk**	**Egg**	**Liver**
Phosphatidylcholine	26–38	24–46	20–29	66–83	43–55
Phosphatidylethanolamine	19–30	21–34	28–36	8–24	23–28
Phosphatidylinositol	12–26	13–21	0–1	0–1	6–9
Phosphatidylserine	traces	5–6	0–8	1–3	3–4
Lysophospholipids	3–9	1–5	traces	3–7	0–2
Sphingolipids	–	–	15–29	1–3	3–5
Phosphatidic acid	0–3	0–14	–	–	0–1

Another way to increase the phosphatidylcholine content in lecithin is enzymatic transesterification after the addition of choline. Alternatively, modification can be achieved by the addition of free fatty acids, which make the product more fluid. Chemical modification of lecithin (hydrogenation and hydroxylation of the double bonds of fatty acids) is used to increase its stability against oxidation. For specific purposes, a phospholipid concentrate (>90% of phospholipids) is produced by a selective extraction that separates neutral lipids, especially triacylglycerols and free fatty acids. The most important product for industrial purposes is soy lecithin. Its composition is shown in Table 3.33. Rapeseed lecithin and sunflower lecithin have quite a different phospholipid fraction composition.

3.5.1.5 Use

The majority of lecithin is used as feed (as extraction meals dipped in hydration sludge) and only a smaller part of a better quality product is used in the food industry, mostly in the bakery industry. Lecithin is used as a substance for improving dough properties, as it increases the amount of gas retained in the rigid dough foam and reduces the rate of amylose retrogradation. It is also used as an emulsifier in the production of mayonnaise and emulsified fats of the margarine type (it allows the creation of both o/w and w/o emulsions), and is used to reduce the viscosity of the chocolate mass in chocolate production. The phospholipid concentrate is used in the production of instant powdered products such as dried milk drinks. The use of lecithin for non-food purposes is very diverse. Previously, a substitute for lecithin was manufactured by phosphorylation of diacylglycerols and subsequent neutralisation of the phosphatidic acids formed (Section 11.5.2.1.1).

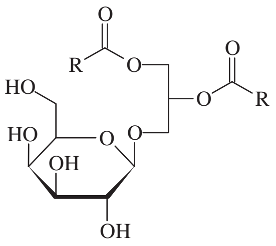
3.5.2 Glycolipids

Glycolipids are fatty acid derivatives that contain bound sugars. If they also contain bound glycerol, they are called glyceroglycolipids, whereas if they contain bound sphingosine, they are called sphingoglycolipids. The most common sugar bound in glycolipids is D-galactose (sometimes several galactose units are linked to each other in one glycolipid molecule), and rarely D-glucose or D-fructose, as well as some other sugars. Glycolipids sometimes contain bound phenolic acids and may then act as antioxidants. Glycolipids accompany phospholipids as a part of cell structures and are also bound in lipoproteins. Reactions of glycolipids are similar to reactions of other heterolipids, but they also exhibit the typical reactions of sugars. The hydrolysis of glycolipids in lecithin yields free sugars that react with the free amino groups of amino

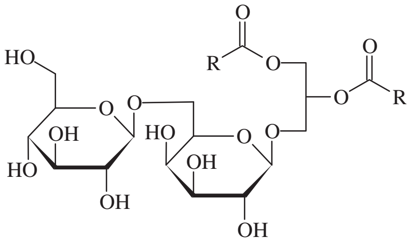
compounds (in the Maillard reaction), which leads to browning of lecithin and formation of undesirable odours.

3.5.2.1 Glyceroglycolipids

Galactolipids (monogalactosyldiacylglycerols and digalactosyldiacylglycerols) are, along with sulfolipids, major components of lipid membranes of chloroplasts and related organelles in photosynthetic organisms (higher plants, algae and some bacteria). The predominant compounds are 1,2-di-*O*-acyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol (**3-71**) and 1,2-di-*O*-acyl-3-*O*-(6′-*O*-α-D-β-galactopyranosyl-D-galactopyranosyl)-*sn*-glycerol (**3-72**). Higher homologues also exist. For example, trigalactosylglycerol has been found in potatoes and tetragalactosylglycerol occurs in oats.[13]



3-71, monogalactopyranosyldiacylglycerol



3-72, digalactopyranosyldiacylglycerol

The main fatty acids of galactolipids in photosynthetic tissues of higher plants are polyene fatty acids, of which α-linolenic acid dominates. Its concentration is up to 95% of total fatty acids. In pea (18:3 plants), α-linolenic acid is practically the only fatty acid bound in positions *sn*-1 and *sn*-2 of monogalactopyranosyldiacylglycerol. The model plant *Arabidopsis thaliana* (16:3 plants) contains exclusively hexadecatrienoic acid bound in the position *sn*-2. Palmitic acid is only found in digalactosyldiacylglycerols, and only in small quantities. Other tissues (tubers, roots and seeds) contain fatty acids with lower number of double bonds. Wheat flour, for example, contains 1.5–2.5% of lipids, of which the smaller part (25%) is bound to starch as the so called starch lipids and the rest are non-starch lipids. The composition of both types of lipids differs significantly. The main components of starch lipids are glycerophospholipids (89.4%) and glyceroglycolipids (5.0%), while triacylglycerols (1.4%) are minor components. The non-starch lipids contain as the major components triacylglycerols (46.7%); other major components are glyceroglycolipids (26.9%) and glycerophospholipids (14.7%). The detailed composition of wheat flour glyceroglycolipids is shown in Table 3.34.

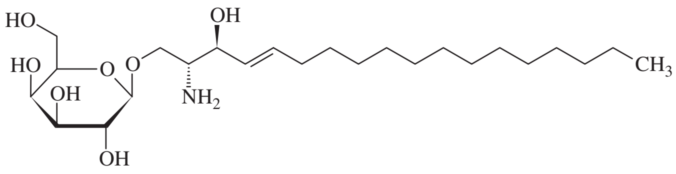
3.5.2.2 Sphingoglycolipids

In addition to *N*-acylsphingosines (ceramides) and sphingophospholipids, sphingosine also occurs in the form of glycosides. These compounds belong to the sphingoglycolipids group. The common sugar bound in sphingoglycolipids is D-galactose. An example of sphingosine galactoside is psychosine (1-*O*-β-D-galactopyranosylsphingosine, **3-73**). Amides of fatty acids (mostly saturated) and sphingosine are glycosides of ceramides (1-*O*-β-D-galactopyranosylceramides, **3-74**), called **cerebrosides**. Also, sphingophospholipids may still contain bound sugar, again mostly galactose.

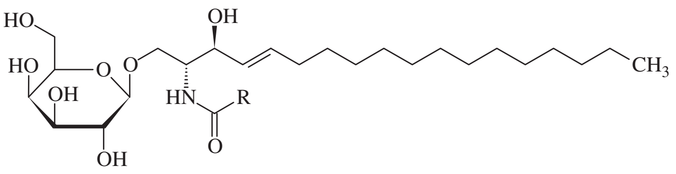
[13]In addition to these glyceroglycolipids, a number of plants and some bacteria contain linear galactolipids in which two to four galactose units are linked by the β-(1→6) glycosidic bond. Oats and rice sprouts contain interesting diacylgalactosylglycerols with an estolide bond composed of 15-hydroxylinoleic acid esterified with linoleic acid and bound to the glycerol *sn*-2 hydroxyl group. The rice germ contains galactolipids together with 1,2-di-*O*-acyl-3-*O*-β-D-glucopyranosyl-*sn*-glycerol, rice seeds also contain triglycosyldiacylglycerols that contain bound galactose and glucose.

Table 3.34 Fatty acid composition of galactolipids of wheat flour (in % of total fatty acids).

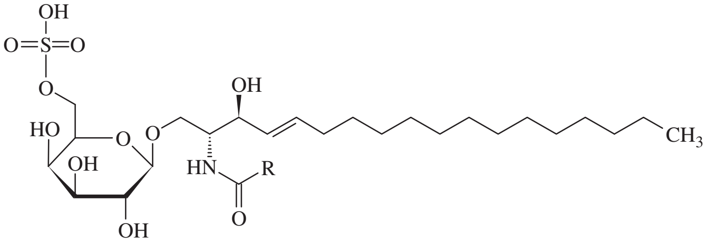
	Fatty acid				
Position in galactolipid	16:0	18:0	9*c*-18:1	9*c*12*c*-18:2	9*c*12*c*15*c*-18:3
Monogalactosyldiacylglycerol					
sn-1	11	1	5	81	1
sn-2	traces	traces	9	83	7
Digalactosyldiacylglycerol					
sn-1	26	2	4	63	4
sn-2	2	traces	7	83	7



3-73, psychosine



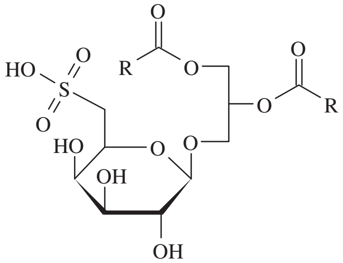
3-74, cerebroside



3-75, glycosylceramide sulfates

3.5.3 Sulfolipids and lipid sulfates

Some heterolipids contain bound sulfuric acid. For example, these heterolipids include **sulfoglycosylsphingolipids**, formerly called sulfatides. For example, members of this lipid group are glycosylceramide sulfates, exemplified by 1-*O*-β-D-galactopyranosylceramide sulfate (**3-75**). Sulfur in some lipids is also bound as sulfonic acid. An examples of these compounds is 2,3-diacyl-1-(6-deoxy-6-sulfo-β-D-galactopyranosyl)glycerol (**3-76**). Sulfolipids and lipid sulfates accompany phospholipids in nature, and are constituents of some complex lipids.



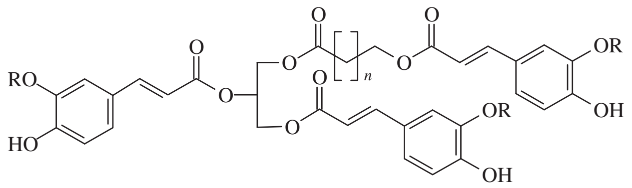
3-76, sulfolipids

3.5.4 Sialolipids

A physiologically important group of lipids is sialoglycosphingolipids, known as gangliosides, which contain bound sialic acid or several sialic acid residues (see Section 4.3.3.7). The level of the gangliosides can reach up to 6% by weight of the brain lipids, where they constitute 10–12% of the total lipids of neuronal membranes. These lipids are mostly oligoglycosphingolipids.

3.5.5 Other heterolipids

Vegetable oils often contain a variety of natural phenolic antioxidants, but usually only as admixtures. Phenolic acids, such as caffeic (R = H) and ferulic ($R = CH_3$) acids, occur in lipids relatively rarely, usually tied to the glycerol residue (**3-77**), and show antioxidant effects.

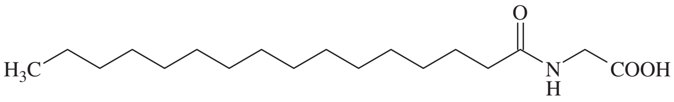


3-77, lipid containing bound phenolic acids (*n* = 24 or 26)

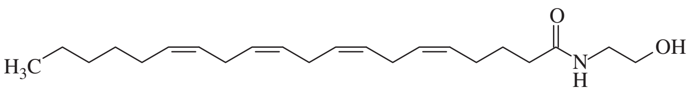
3.6 Miscellaneous simple and complex lipids

3.6.1 Lipoamino acids and fatty acid amides

Many different simple fatty acyl-amino acids also known as lipoamino acids are present in animal tissues and as constituents of bacterial lipids. Examples of simple lipoamino acids are *N*-palmitoylglycine (**3-78**) and *N*-oleoylglycine, which have roles in sensory neuronal signalling and regulation of body temperature and locomotion, respectively. *N*-Arachidonylglycine has been shown to suppress inflammatory pain. Biologically active compounds are also other other long-chain *N*-acylethanolamides, for example, *N*-palmitoylethanolamide and *N*-oleoylethanolamide, or oleamide.



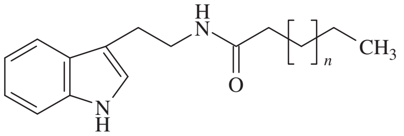
3-78, *N*-palmitoylglycine



3-79, anandamide

Some fatty acid amides that occur naturally can have profound biological functions. For example, *N*-acylethanolamides, such as *N*-arachidonoylethanolamide (anandamide, **3-79**), are ubiquitous trace constituents of animal and human cells, tissues and body fluids. The name is taken from the Sanskrit word ananda, which means bliss or delight. Anandamide is an endogenous cannabinoid neurotransmitter that occurs naturally in the brain and in some foods (as chocolate) and that binds to the same brain receptors as the cannabinoids (as tetrahydrocannabinol).

Fatty acid tryptamides (**3-80**) can be employed as indicator substances for detecting the shell content in cocoa products. The shell content is an important quality parameter for products that are made from roasted cocoa beans. The relevant substances are docosanoyl-2-(3-indolyl)ethylamide (behenic acid tryptamide) and tetracosanoyl-2-(3-indolyl)ethylamide (lignoceric acid tryptamide). Cocoa shells contain 330–395 mg/kg behenic acid tryptamide and lignoceric acid tryptamide, but the cotyledons only 7–10 mg/kg.



3-80, fatty acid tryptamides
behenic acid tryptamide, $n = 17$
lignoceric acid tryptamide, $n = 19$

3.6.2 Complex lipids

Complex lipids are macromolecular substances whose lipid constituent is bound to the non-lipid components by hydrogen bonds, hydrophobic interactions and other physical bonds, but some may also be bound by covalent bonds. The non-lipid components are mostly proteins, but also polysaccharides, mixtures of protein with polysaccharides, lignin and other substances.

3.6.2.1 Lipoproteins

Lipoproteins are the most important and best researched complex lipids, and are composed of proteins and lipids. Lipids often form the core of macromolecules while the hydrated proteins form the coat. Therefore, lipoproteins dissolve (or at least are dispersed) in water, and serve to transport lipids. Lipoproteins usually consist of non-polar lipids, such as triacylglycerols or cholesterol esters, cholesterol and polar lipids (such as phospholipids), which facilitate the link between lipids and proteins. The best examined lipoproteins are serum lipoproteins, because they have great significance in the development of circulatory diseases. Historically, serum lipoproteins were divided according to their specific gravity (density). When lipoproteins contain more non-polar lipids their density is lower, and vice versa. The lower the density of lipoproteins, the weaker and less complex is the protein coat, which is maintained in an aqueous solution (Figure 3.7 and Figure 3.8). To date, five major groups of lipoproteins are recognised (Table 3.35):

- **chilomicrons**
- **very low density lipoproteins** (VLDL)
- **intermediate density lipoproteins** (IDL)
- **low density lipoproteins** (LDL)
- **high density lipoproteins** (HDL).

Chylomicrons are particles (100–1000 nm in diameter) that carry the ingested lipids (triacylglycerols) from the intestinal wall into the tissues (adipose tissue and muscles), where they are stored, while VLDL (30–80 nm) carry the newly synthesised triacylglycerols from the liver to adipose tissue. Milk fat globules (see Section 2.4.5.2) have a similar structure to chilomicrons. IDL (the degradation products of VLDL; 25–50 nm) circulate through the body and

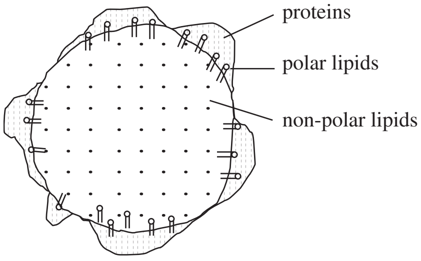


Figure 3.7 Schematic composition of low density lipoproteins.

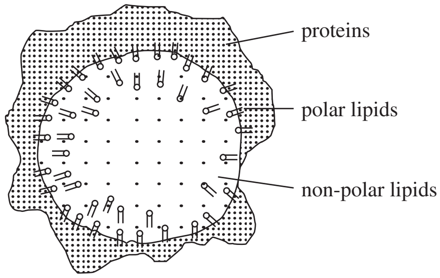


Figure 3.8 Schematic compositions of high density lipoproteins.

Table 3.35 Overview of serum lipoproteins.

Lipoprotein type[a]	Density (kg/l)	Triacylglycerols (%)	Cholesterol/its esters (%)	Phospholipids (%)	Proteins (%)
Chylomicrons	<0.95	85-88	1/3	8	1-2
VLDL	0.95-1.006	50-55	8-10/12-15	18-20	7-10
IDL	1.006-1.019	25-30	8-10/32-35	25-27	10-12
LDL	1.019-1.063	10-15	8-10/37-48	20-28	20-22
HDL	>1.063	3-15	2-10/15-30	32-43	33-57

[a]VLDL = Very low density lipoprotein, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, HDL = high density lipoprotein.

transport cholesterol. They also have the ability to promote plaque formation in vessels. LDL (18–28 nm) carry cholesterol from the liver to the body cells, while HDL (5–15 nm) carry cholesterol from the body cells to the liver. The LDL are much less stable than the HDL. Lipids from lipoproteins are readily cleared and temporarily stick to the walls of blood vessels.

Lipoproteins are also components of membranes, forming structures with bilayers of oriented molecules of polar lipids (Figure 3.9). Owing to their polar character induced by the double layer of lipids with an intermediate layer of protein, they are a suitable barrier for the transport of substances between cells or within individual intracellular structures. These properties are also used in the pharmaceutical industry for emulsifying fats with proteins to form liposomes that facilitate the transfer and absorption of certain medications. The creation of the liposomes can explain some changes in the properties of foods during processing. Reactions of lipoproteins involve all lipoprotein components, lipids and proteins. Protein reactions with free radicals yield modified structures, where the lipid part is tightly bound to protein. These structures are thought to be the major components of aterosclerotic deposits in blood vessels. Reactions with peroxyl radicals, which are derived from unsaturated lipids, lead to insoluble macromolecular brown coloured compounds called **ceroids** or **stichosterins** that are deposited in the walls of blood vessels and other places in the body such as the kidneys or uterus. Similar structures are found in deposits in nerve tissue, and are called age pigments (see Section 4.7.5.6). This granular yellow–brown pigment is called **lipofuscin**.[14]

3.6.2.2 Mucolipids

Significant mucolipids are gangliosides containing bound sialic acid that are present in neural tissues. The individual components are bound by covalent bonds, but also by physical links. Examples of gangliosides are listed in Table 3.36.

[14]Similar substances have been reported in animals fed with oxidised fish meal that caused the so called yellow fat disease accompanied by animal mortality. This pigment was first observed in the brain and other light coloured tissues of turkeys.

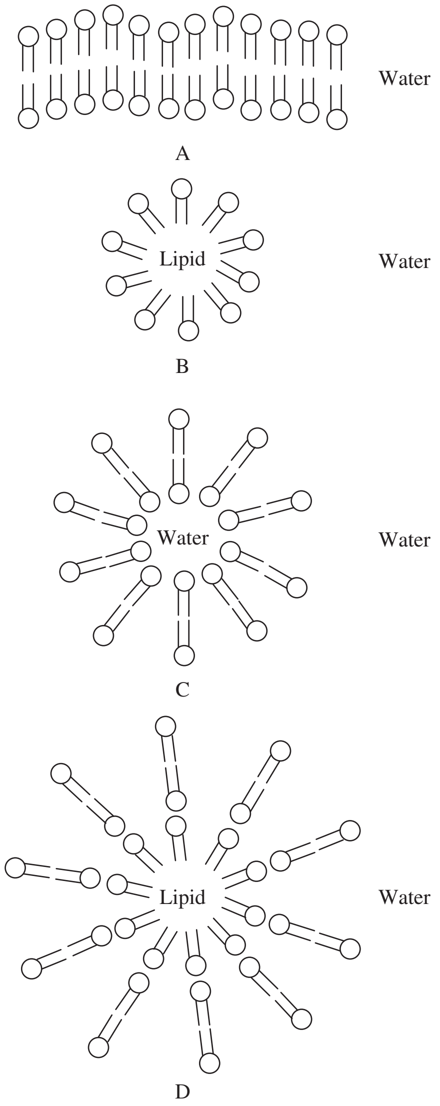


Figure 3.9 Schematic illustrations of lipid membranes in food materials: A = double lipid cell membrane, B = nanoparticles bound with simple lipid membrane with lipid phase within the particles, C = liposome bound by double lipid membrane with the aqueous phase inside the particle, D = fat particles stabilised by lamella structure bound by a double lipid membrane and by simple lipid membrane with fat phase inside the particle.

Table 3.36 Composition of gangliosides.[a]

Sialic acid units	Oligosaccharide	Ceramide	Sialic acid units	Oligosaccharide	Ceramide
II^3-NeuAc	Lac	Cer	V^3-NeuAc	GgO_5	Cer
II^3-NeuAc	triaose	Cer	II^3NeuGc	nLacO_4	Cer
IV^3-NeuAc_2	GgO_4	Cer	V^3NeuGc	iGgO_5	Cer

[a]NeuAc = acetylneuraminic acid, NeuGc = glycolylneuraminic acid; roman numerals at the beginning = bond position in sugar; exponent = to which oligosaccharide unit sialic acid is bound; index at the end = number of sialic acid units bound in a row; Lac = lactose; triaose (three sugar units); GgO = globoose, oligosaccharide with any sugar units, *n* = neobond (1→3); *i* = isobond (1→4); index at the end = number of sugar units, Cer = residue of any sphingoid base (generally identified as ceramide).

3.6.2.3 Lipid clathrates

Clathrates (cage or inclusion compounds)[15] are compounds that consist of a lattice of one type of molecule (a host molecule) trapping another type of molecule (a guest molecule) by intermolecular interactions. A clathrate is therefore a mechanical structure without chemical bonds. Such compounds include protein complexes containing β-carotene or lipids (fatty acids) bound in the starch macromolecules (see Section 4.5.1.1). In some technological processes, such as extrusion, part of the free lipids is bound in this way.

3.7 Substances accompanying lipids

Food raw materials and products contain, in addition to lipids, many lipophilic compounds sometimes called **lipoids**, which during the isolation of lipids pass into the lipid fraction due to its low polarity. They are therefore called lipid accompanying substances, although this does not necessarily mean that they accompany lipids in the original material or that they are somehow functionally related to lipids. The lipid accompanying substances include higher hydrocarbons, higher primary and secondary alcohols, ketones and diketones, various steroids, lipophilic vitamins, pigments and other lipophilic compounds specific to certain materials.

Lipophilic polycyclic aromatic hydrocarbons (see Section 12.2.5), condensed aromatic heterocycles (see Section 12.2.1) and other lipophilic compounds formed in processed foods or compounds derived from external sources are not considered to be accompanying substances of lipids. They are classified as food contaminants.

3.7.1 Hydrocarbons

Higher hydrocarbons are found mainly in waxes, where they can form as much as a few percent of the weight of the wax, but they can also be found in small quantities in common edible fats and oils. They come mainly from the waxy surface layer of seeds. The majority of hydrocarbons have 15–35 carbon atoms in the molecule. The most frequently occurring hydrocarbons are alkanes (*n*-alkanes) with an odd number of carbon atoms. Less common are isoalkanes (mainly with an odd number of carbon atoms) and ante-isoalkanes (mainly with an even number of carbon atoms). Sometimes higher alkenes are also present. Qualitative and quantitative composition of hydrocarbons is often characteristic of certain foods and can be used to identify them. For example, the surface waxes of fruits and oilseeds, and therefore also most food oils, contain alkanes C_{27}, C_{29} and C_{31} as major components, but olive oil is dominated by alkanes C_{23}, C_{25}, C_{27} and C_{29} (Table 3.37).

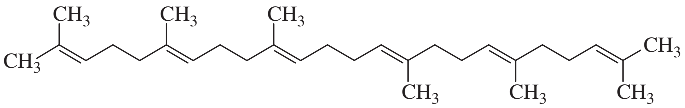
The total alkane contents in vegetable oils ranges from about 10 to 200 mg/kg. Their amount in sunflower oil is 105–170 mg/kg, and in olive oil approximately 30–100 mg/kg. The amount of alkanes in the cuticular wax of apples, for instance, is about 33% of the lipid accompanying substances. About 97% of all hydrocarbons in the cuticular wax are represented by nonacosane (C_{29}), about 2% by heptacosane (C_{27}) and the remaining 1% consists mainly of alkanes C_{26}, C_{28}, C_{30} and C_{31}. The cuticular wax of grapefruits is composed primarily of oxygenated compounds, while the content of alkanes is only around 1%. Fats of marine organisms (e.g. fish liver oils) contain saturated and unsaturated hydrocarbons C_{15} and C_{17} or C_{21} as the main components. The total content of alkanes is about 10–30 mg/kg.

In addition to the above mentioned hydrocarbons, oils also contain alkenes and monoterpenic and sesquiterpenic hydrocarbons. For example, the amount of alkenes in olive oils range from 0.5 to 2 mg/kg and includes a series of alk-9-enes from C_{22} to C_{27}, heptadec-8-ene and 6,10-dimethyl-undec-1-ene. An important compound is the linear triterpenic (C_{30}) hydrocarbon squalene (**3-81**), all-*trans*-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene, which is the universal precursor of all triterpenoids and steroids. It was given this name because it was discovered in the liver of sharks (Squalidae). Shark liver oil contains about 30% squalene, about 7% pristane (2,6,10,14-tetramethylpentadecane) and smaller amounts of phytane (2,6,10,14-tetramethylhexadecane). Squalene occurs in small amounts in edible oils and especially in olive oil, where its content is in the tenths of a percent (1–7 g/kg).

[15]The most famous clathrates are clathrates of lipids with urea. Urea forms a cylindrical structure, formed by spiral molecules bound by hydrogen bonds, which entrap fatty acid or other derivatives with a long unbranched saturated chain (e.g. hydrocarbons). Branched or *cis*-unsaturated fatty acids bind much less tightly, triacylglycerols do not form clathrates with urea, because their volume is bigger and the molecules do not fit into the housing formed by urea molecules.

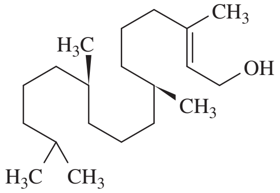
Table 3.37 Content of alkanes in sunflower and virgin olive oils.

Alkane	Name	Content (mg/kg oil)		Alkane	Name	Content (mg/kg oil)	
		Sunflower	Olive			Sunflower	Olive
C_{16}	Hexadecane	0.13	0.06	C_{25}	Pentacosane	1.52	17.98
C_{17}	Heptadecane	0.16	0.12	C_{26}	Hexacosane	0.41	2.04
C_{18}	Octadecane	0.90	0.08	C_{27}	Heptacosane	11.19	15.72
C_{19}	Nonadecane	0.12	0.13	C_{28}	Octakosane	2.38	1.84
C_{20}	Eicosane	0.02	0.08	C_{29}	Nonacosane	49.63	12.38
C_{21}	Heneicosane	0.04	0.81	C_{30}	Triacontane	5.52	1.70
C_{22}	Docosane	0.04	1.24	C_{31}	Hentriacontane	47.96	9.41
C_{23}	Tricosane	0.15	18.54	C_{32}	Dotriacontane	1.79	1.54
C_{24}	Tetracosane	0.17	9.54	C_{33}	Tritriacontane	3.60	5.66



3-81, squalene

In addition to the previoulsy mentioned less volatile higher hydrocarbons, hydrocarbons with a short chain, such as pentane and hexane, may also be found in fats. They form by cleavage of fatty acid hydroperoxides produced by oxidation of unsaturated fatty acids. These hydrocarbons, not ranked among the lipid accompanying compounds, are easily removed by heating, so they do not occur in freshly refined oils.



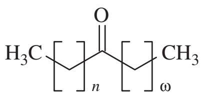
3-82, phytol

3.7.2 Aliphatic alcohols

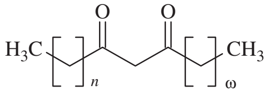
Aliphatic primary alcohols C_{12}–C_{36} accompany waxes, but may also occur in trace amounts in ordinary cooking oils, where they pass from the surface layers during seed extraction. Their structure therefore corresponds to the alcohols bound in waxes. For example, the major components of the wax on the surface of sunflower seeds (and of apple wax), along with a number of minor alcohols, are the alcohols C_{24}, C_{26}, C_{28} and C_{30}. In addition to these higher alcohols, called fatty alcohols, vegetable oils and animal fats also contain lower primary alcohols with 3–11 carbon atoms, which are formed from hydroperoxides by the hydrocarbon chain cleavage. In addition to primary alcohols, higher secondary alcohols also occur in vegetable oils. Their chain length corresponds to the alkanes from which they arise by oxidation. Their hydroxyl group is located near the centre of the hydrocarbon chain. A common secondary alcohol is nonacosan-15-ol. Vegetable oils may also contain traces of the diterpenic alcohol phytol (**3-82**), (2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol, which is, for example, bound in chlorophyll dyes (see Section 9.2.2). Free alcohol arises during catabolic processes catalysed by chlorophyllase. Diterpenes, however, are not included as lipid accompanying substances.

3.7.3 Aliphatic ketones

Higher ketones (**3-83**), usually C_{24}–C_{33} compounds, arise as products of oxidation of alkanes via secondary alcohols. Higher β-diketones, mainly C_{31} and C_{33} compounds (**3-84**), also accompany higher ketones in smaller amounts.



3-83, aliphatic ketones



3-84, aliphatic β-diketones

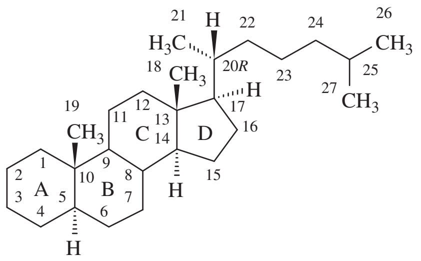
3.7.4 Triterpenoids and steroids

Triterpenoids and steroids are the major group of the lipid-accompanying substances in all natural lipids. Triterpenoids and steroids belong to a large group of compounds known as **terpenoids** or **isoprenoids** (through the oxidation or rearrangement modified terpenes). Six isoprene (2-methylbuta-1,3-diene) units

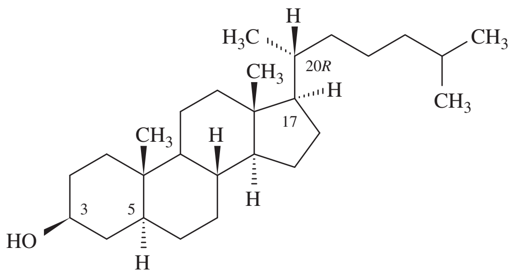
give **triterpenes** with 30 carbon atoms and their modifications yield **triterpenoids**. Several thousand natural triterpenoids include compounds with nearly 200 types of skeletons. **Steroids** are a group of terpenoids arising from triterpenoid precursors. Some steroids play a number of vital functions in living organisms. Examples of such compounds are the steroid hormones of animals, plant steroid hormones brassinoids, steroid glycoalkaloids (see Section 10.2.3.1.8), phytoanticipins and phytoalexins. Some compounds have found use in medicine (such as cardioactive steroid glycosides).

3.7.4.1 Structure and nomenclature

Triterpenoids are mostly C_6–C_6–C_5–C_6 tetracyclic or C_6–C_6–C_6–C_6–C_5 and C_6–C_6–C_6–C_6–C_6 pentacyclic compounds, but there are also acyclic, monocyclic, bicyclic, tricyclic and hexacyclic triterpenoids. Triterpenoids with a β-hydroxyl group at C-3 and a skeleton derived theoretically from the hydrocarbon α-cholestane (**3-85**), such as tetracyclic cyclopenta[*a*]phenanthrene, are structures with a (20*R*)-configuration and are called **steroids**. Almost all steroids are, in addition to 5α-cholestan-3β-ol (**3-86**), secondary alcohols with a perhydro-1,2-cyclopentanophenanthrene C_6–C_6–C_6–C_5 skeleton, which consists of three six-membered rings A, B and C in a nonlinear arrangement, with the circle C connected to a five-membered ring D.[16] The steroid skeleton contains a secondary hydroxy group in the C-3 position of ring A, therefore sterols are actually alicyclic alcohols. The positions C-10 and C-13 always contain methyl groups and the position C-17 contains a side chain with 8–10 carbon atoms. All of these substituents are in the β-position. Individual steroids vary in carbon number and the number and location of double bonds in the side chain attached to C-17. In addition, steroids differ in substituents at C-4 (hydrogen, one or two methyl groups), number and location of double bonds in ring B and also in the stereochemistry of some asymmetric centres. Some related compounds contain the fifth ring E formed by cyclisation of the side chain that is connected to ring D. These compounds are found, for example, as aglycones of many saponins (see Section 10.3.2.2).



3-85, 5-cholestane



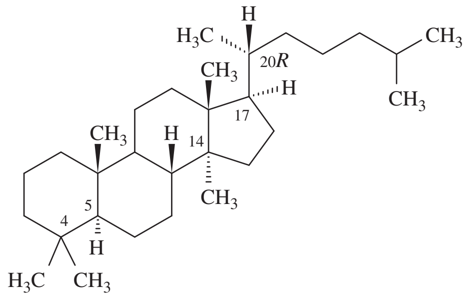
3-86, 5α-cholestan-3β-ol

Most steroids have trivial names, usually created by the Latin or Greek name of the material from which they were isolated. For less common steroids, systematic names are used, which are derived from basic structures. In addition to 5α-cholestane (**3-85**), other basic structures of hydrocarbons are 5α-lanostane (**3-87**), 5α-ergostane (**3-88**), 5α-campestane (**3-89**), 5α-poriferastane (**3-90**) and 5α-stigmastane (**3-91**), which differ in the structure of the side chain at C-17. The classification reflecting the biochemical origins distinguishes three groups of steroids by the number of methyl groups on carbon C-4. The recognised groups are:

- **4.4-dimethylsterols**
- **4-methylsterols**
- **4-demethylsterols** (as methyl groups in position 4 is missing) simply called sterols.

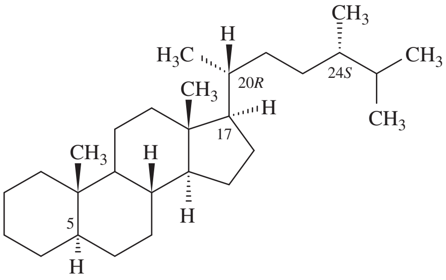
Steroids of the first two groups that contain 30 carbon atoms are also known as **triterpenic alcohols**. This name is often used for all 4,4-dimethylsterols and 4-methylsterols. Both types of steroids may, however, in addition to 4-demethylsterols, contain less or more than 30 carbon atoms in the molecule.

An overview of significant compounds is given in Table 3.38. Lanosterol, for example, is 4,4,14-trimethyl-5α-cholesta-8,24-diene-3β-ol or 5α-lanosta-8,24-diene-3β-ol. The hydrocarbon

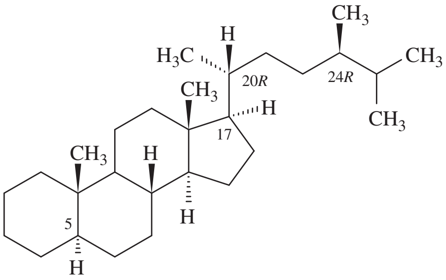


3-87, 5-lanostane

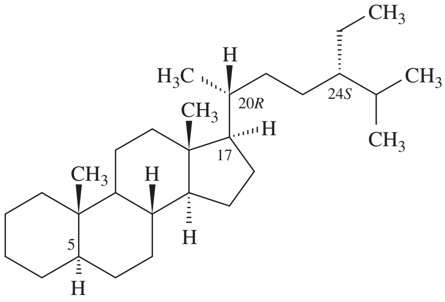
[16]Numbers 28, 29 and 30 are reserved for methyl groups of triterpenoids at C-4 and C-14. The basic structure has eight chiral centres (carbon atoms C-3, C-5, C-8, C-9, C-10, C-13, C-14 and C-17) and another in the side chain at C-17. Most natural steroids have the same basic structures. The circles AB, BC and CD are always in the *trans*-position. The steroid molecule is nearly a planar unit and individual substituents lie above or below this plane. The orientation point is a methyl group at C-10, which is always above the plane. Substituents that are in the *cis*-position to this methyl group (they also lie above the plane), are β-substituents, while the substituents in *trans*-position are called α-substituents. The name must always indicate the position of hydrogen on C-5, which in this case is in the 5α position.



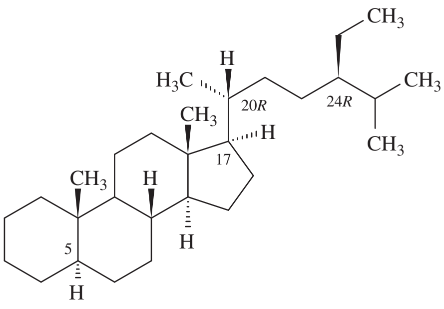
3-88, 5-ergostane



3-89, 5-campestane



3-90, 5-poriferastane

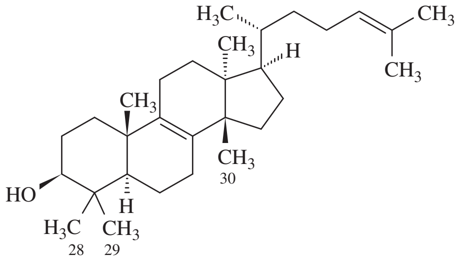


3-91, 5-stigmastane

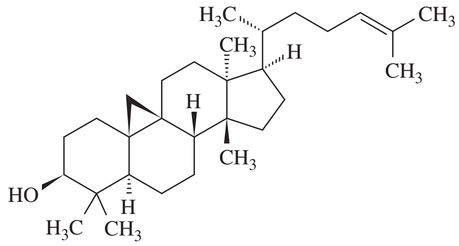
lanostane (**3-87**), from which lanosterol is derived, is 4,4,14-trimethyl-5α-cholestane. Analogously, cycloartenol is 4,4,14-trimethyl-9,19-cyclo-5α,9β-cholesta-24-en-3β-ol or 9,19-cyclo-5α-lanost-24-en-3β-ol, campesterol is (24*R*)-24-methylcholesta-5-en-3β-ol or campest-5-en-3β-ol (**3-89**), stigmasterol is (24*S*)-24-ethylcholesta-5,22-dien-3β-ol or (*E*)-stigmasta-5,22-dien-3β-ol (**3-91**), β-sitosterol is (24*R*)-24-ethylcholest-5-en-3β-ol or stigmasta-5-en-3β-ol (**3-91**), ergosterol is (22*E*,24*R*)-24-methyl-cholesta-5,7,22-trien-3β-ol or (*E*)-ergosta-5,7,22-trien-3β-ol (**3-88**).

3.7.4.1.1 Dimethylsterols

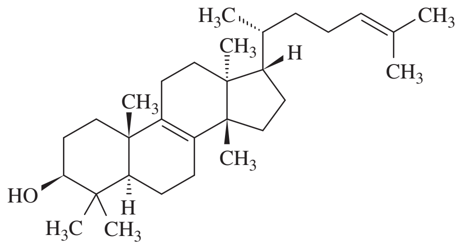
The basic 4,4-dimethylsterols are C_{30} steroids lanosterol (**3-92**) and cycloartenol with a cyclopropane ring (**3-93**), derived from lanostane (4,4,14-trimethylcholestane). Lanosterol is the building block for the biosynthesis of all other zoosterols (including cholesterol), the biosynthesis of many other phytosterols is based on cycloartenol. Common compounds are also euphol (**3-94**) and its isomer butyrospermol (Δ^7-euphol or Δ^7-tirukallol, **3-95**) derived from the hydrocarbon euphane.



3-92, lanosterol



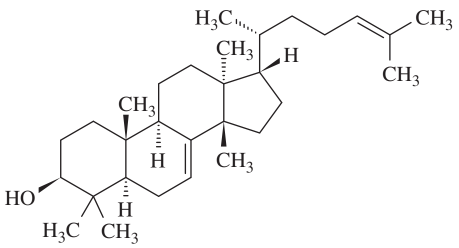
3-93, cycloartenol



3-94, euphol

Table 3.38 Overview of trivial and systematic names of selected steroids.

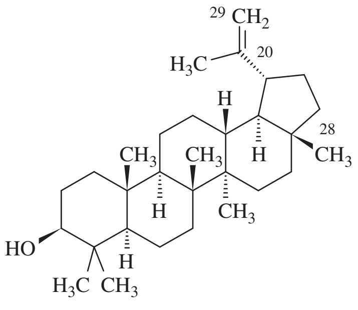
Steroid name	
Trivial	**Systematic**
4,4-Dimethylsterols	
α-Amyrin	5α-Urs-12-en-3β-ol
β-Amyrin	5α-Olean-12-en-3β-ol
Betulinic Acid	5α-Eupha-7,24-dien-3β-ol-28-carboxylic acid
Butyrospermol	5α-Eupha-7,24-dien-3β-ol
Cycloartenol	9,19-Cyclo-5α-lanost-24-en-3β-ol
Cyclobranol	24-Methyl-9,19-cyclo-5α-lanost-24-en-3β-ol
Cyclolaudenol	(24*S*)-24-Methyl-9,19-cyclo-5α-lanost-25-en-3β-ol
Cyclosadol	24-Methyl-9,19-cyclo-5α-lanost-23-en-3β-ol
Erythrodiol	5α-Olean-12-en-3β,28-diol
Euphol	5α-Eupha-8,24-dien-3β-ol
Lanosterol	5α-Lanosta-8,24-dien-3β-ol
Lupeol	5α-Lup-20(29)-en-3β-ol
24-Methylenecycloartanol	24-Methylene-9,19-cyclo-5α-lanostan-3β-ol
Oleanolic acid	5α-Olean-12-en-3β-ol-28-carboxylic acid
Parkeol	5α-Lanosta-9(11),24-dien-3β-ol
Ursolic acid	5α-Urs-12-en-3β-ol-28-carboxylic acid
Uvaol	5α-Urs-12-en-3β,28-diol
4-Methylsterols	
Citrastadienol	4α-Methyl-24-ethylidene-5α-cholest-7-en-3β-ol
Cycloeucalenol	4α,14α-Dimethyl-24-methylene-9β,19-cyclo-5α-cholestan-3β-ol
Gramisterol	4α-Methyl-24-methylene-5α-cholest-7-en-3β-ol
Lophenol	4α-Methyl-5α-cholest-7-en-3β-ol
Obtusifoliol	4α,14α-Dimethyl-24-methylene-5α-cholest-8-en-3β-ol
4-Demethylsterols	
Δ^5-Avenasterol	(*Z*)-Stigmasta-5,24(28)-dien-3β-ol
Δ^7-Avenasterol	(*Z*)-Stigmasta-7,24(28)-dien-3β-ol
Brassicasterol	(*R*)-24-Methylcholesta-5,22-dien-3β-ol
Desmosterol	Cholesta-5,24-dien-3β-ol
Fucosterol	(*E*)-Stigmasta-5,24(28)-dien-3β-ol
Cholesterol	Cholest-5-en-3β-ol
Campesterol	(*R*)-24-methylcholest-5-en-3β-ol
Clerosterol	(*Z*)-Stigmasta-5,25(26)-dien-3β-ol
Lathosterol	Cholest-7-en-3β-ol
Lichesterol	(*Z*)-Ergosta-5,8,22-trien-3β-ol
Poriferasterol	(*E*)-Stigmasta-5,22-dien-3β-ol
β-Sitosterol	(*R*)-24-Ethylcholest-5-en-3β-ol or stigmasta-5-en-3β-ol
Spinasterol	(*Z*)-Stigmasta-5,22-dien-3β-ol or (24*R*)-24-ethylcholesta-5,22-dien-3β-ol
Stigmasterol	(*S*)-24-Ethylcholesta-5,22-dien-3β-ol or (22*E*)-stigmasta-5,22-dien-3β-ol
Ergosterol	(*E*)-Ergosta-5,7,22-trien-3β-ol or (24*R*)-24-methylcholesta-5,7,22-trien-3β-ol



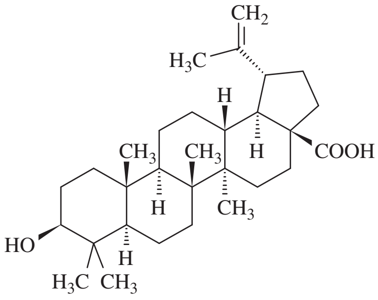
3-95, butyrospermol

Steroids (C_{30}) with four C_6 cycles and one C_5 cycle E, which are derived from the hydrocarbon lupane, are lupeol (**3-96**) and a product of its oxidation on C-28 known as betulinic acid (**3-97**). C_{30} Steroids also occur with five C_6 cycles. The most important of these compounds are α-amyrin (**3-98**), derived from the hydrocarbon ursane, and β-amyrin (**3-99**), which is derived from the hydrocarbon oleane. Also common is taraxasterol (**3-100**), which is structurally related to α-amyrin. Fruit waxes very often contain terpenoid (steroid) C_{30} diols and acids formed by oxidation of the C-27 methyl group. Determination of uvaol (**3-101**) and erythrodiol (**3-102**) concentrations can be used to distinguish olive oils from other vegetable oils. Widespread in cuticular wax of apples, pears, grapefruits and other fruits is ursolic acid (**3-103**), which is structurally related to α-amyrin. Some citrus fruits contain oleanolic acid (**3-104**), related to β-amyrin, in significant quantities. These substances, correspondingly derived saponins (see Section 10.3.2.2), show a number of interesting physiological effects.

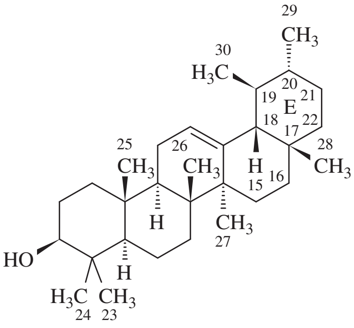
The common C_{31} 4,4-dimethylsterols are represented by isomeric compounds with a cyclopropane ring, such as cycloartenol and 24-methylenecycloartanol (**3-105**), cyclosadol (**3-106**), cyclobranol (**3-107**) and cyclolaudenol (**3-108**), which are derived from the hydrocarbon lanostane.



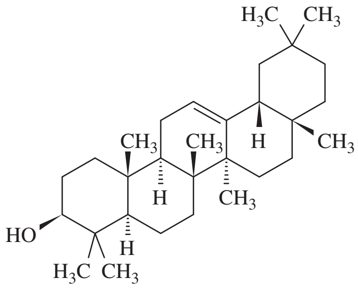
3-96, lupeol



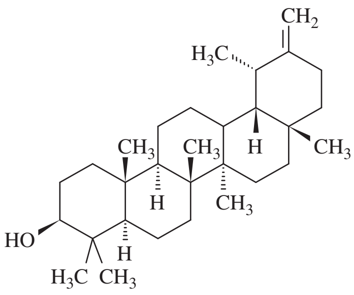
3-97, betulinic acid



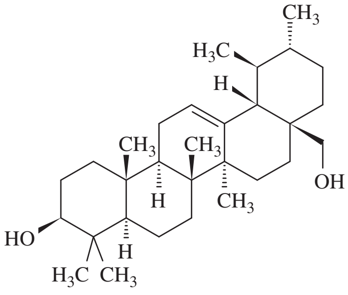
3-98, α-amyrin



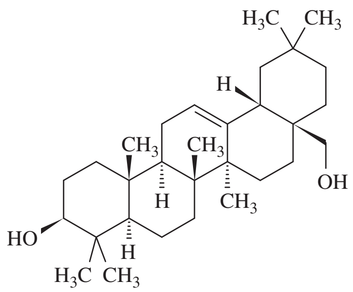
3-99, β-amyrin



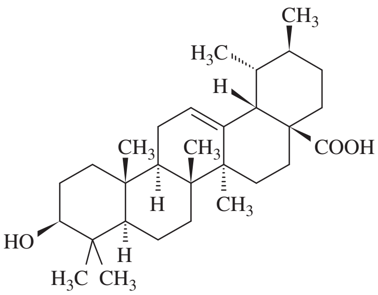
3-100, taraxasterol



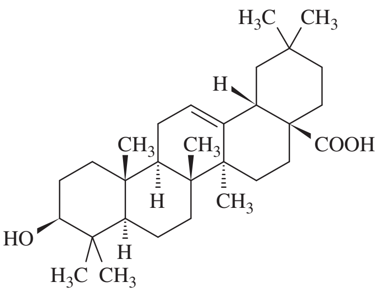
3-101, uvaol



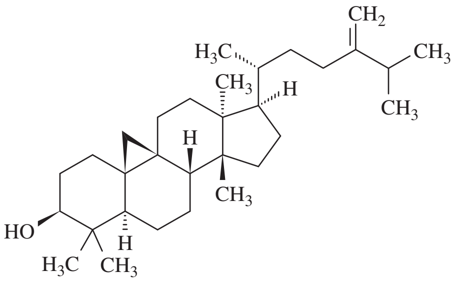
3-102, erythrodiol



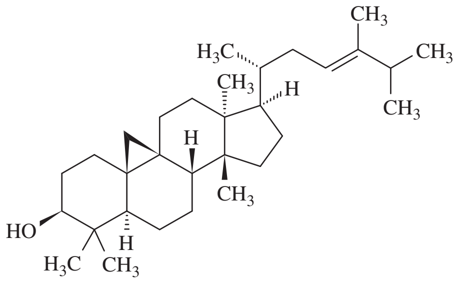
3-103, ursolic acid



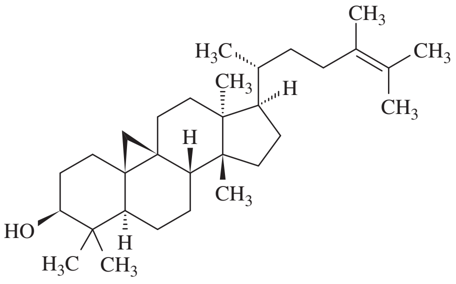
3-104, oleanolic acid



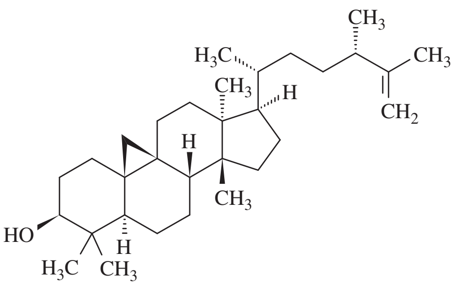
3-105, 24-methylenecycloartanol



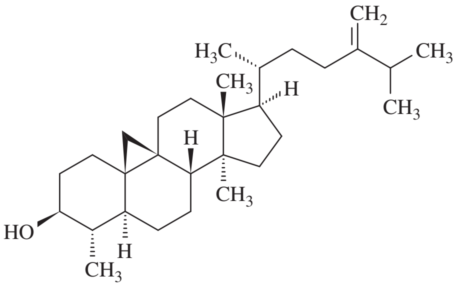
3-106, cyclosadol



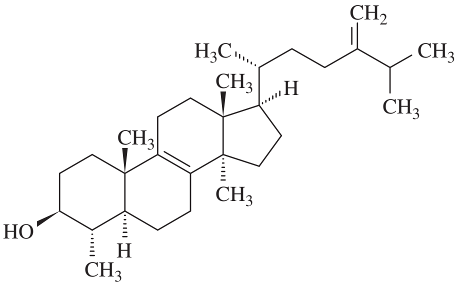
3-107, cyclobranol



3-108, cyclolaudenol



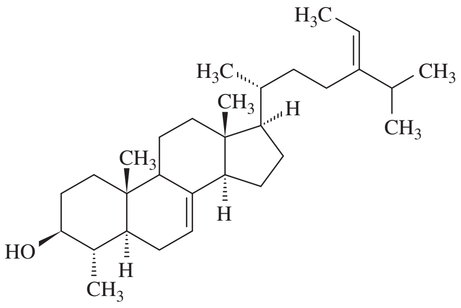
3-109, cycloeucalenol



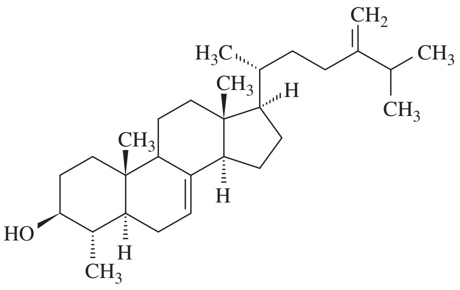
3-110, obtusifoliol

3.7.4.1.2 Methylsterols

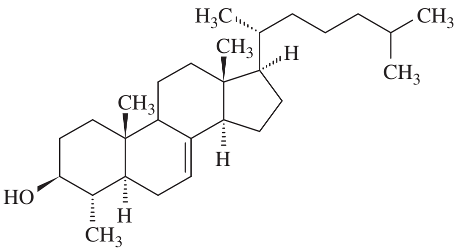
Typical 4-methylsterols are the C_{30} compounds cycloeucalenol (**3-109**) and obtusifoliol (**3-110**), which are intermediates in the biosynthesis of phytosterols from cycloartenol. Other C_{30} compounds include citrastadienol (24-ethylidenelophenol, **3-111**), which is the intermediate in the biosynthesis of β-sitosterol and stigmasterol. The C_{29} compounds are represented by gramisterol (episterol, **3-112**) and C_{28} compounds by lophenol (**3-113**).



3-111, citrastadienol



3-112, gramisterol



3-113, lophenol

3.7.4.1.3 Sterols

Sterols (demethylsterols) are still commonly divided into categories according to their occurrence in nature. The recognised categories are:

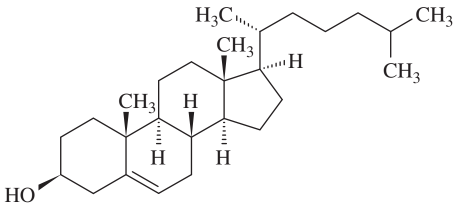
- **zoosterols** (animal sterols)
- **phytosterols** (plant sterols)
- **mycosterols** (sterols of fungi).

This classification has a number of weaknesses. Some bacteria, for example, accept the zoosterol cholesterol from the host animals and use it as a component of membranes, some types of prokaryotic organisms even synthesise sterols *de novo*, some eubacteria (species of the genus *Methylobacterium* and *Methylosphaera*) synthesise 4-methylsterols and 4,4-dimethylsterols (including lanosterol). In many species of bacteria the so-called hopanoids, pentacyclic

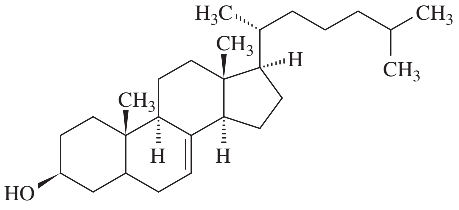
triterpenoids with the $C_6–C_6–C_6–C_5–C_6$ skeleton of the hydrocarbon hopane (with cyclopentane ring E) play the role of sterols.

Modern classification is based on the structure of the sterols. The most frequently occurring compounds are sterols with a double bond in position C-5 in ring B (Δ^5-sterols) and a saturated or unsaturated side chain at position C-17, less common are Δ^7- and $\Delta^{5,7}$-sterols. In plants, these sterols are accompanied by small quantities of saturated sterols known as phytostanols that are analogues of Δ^5-sterols without the double bond in ring B. They occur in cereals in relatively high amounts.

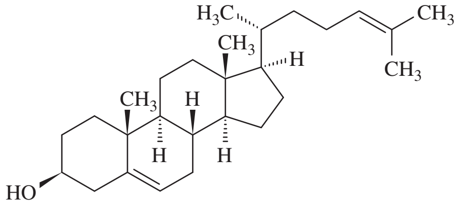
The basic C_{27} sterol is cholesterol, cholesta-5-en-3β-ol or (3β)-cholest-5-en-3-ol (**3-114**), with a saturated C_8 side chain. The same side chain also occurs in its precursor lathosterol (**3-115**); unsaturated C_8 side chain has its other precursor desmosterol (**3-116**). Normally, there are also sterols with 28 carbon atoms in the molecule. Their representatives are the Δ^5-sterols campesterol (**3-117**) and brassicasterol (**3-118**). The most common C_{29} Δ^5-sterol is sitosterol (also called β-sitosterol, **3-119**). Also found, at lower levels, are stigmasterol (**3-120**), avenasterol (or Δ^5-avenasterol or 5-avenasterol, **3-121**), clerosterol (**3-122**), poriferasterol, fucosterol (**3-123**) and Δ^7-sterols, such as spinasterol (**3-124**), Δ^7-campesterol, Δ^7-avenasterol and Δ^7-stigmasterol. An important $\Delta^{5,7}$-sterol is ergosterol (**3-125**).



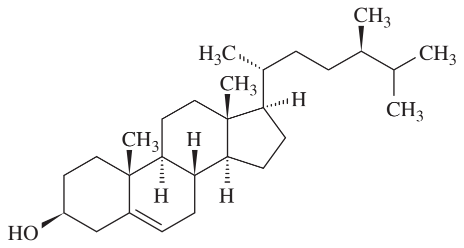
3-114, cholesterol



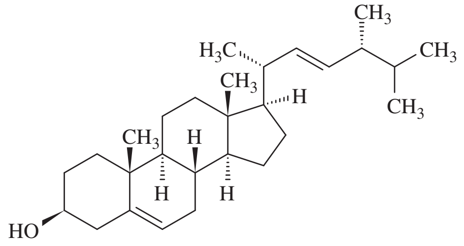
3-115, lathosterol



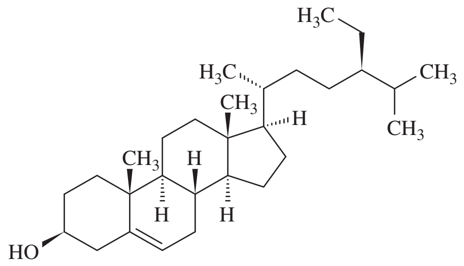
3-116, desmosterol



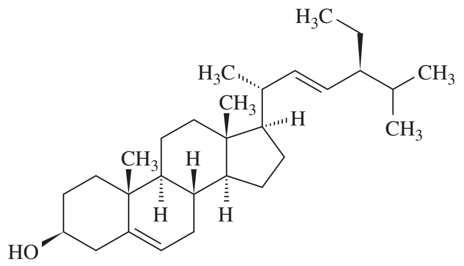
3-117, campesterol



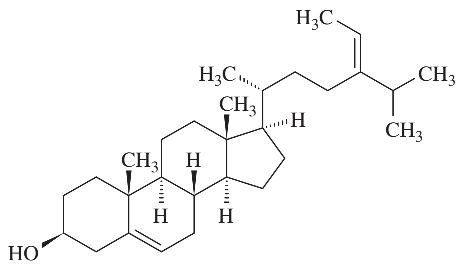
3-118, brassicasterol



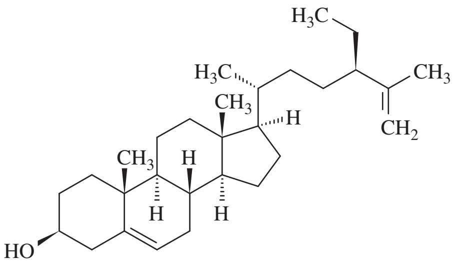
3-119, β-sitosterol



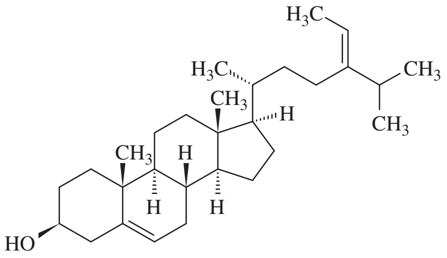
3-120, stigmasterol



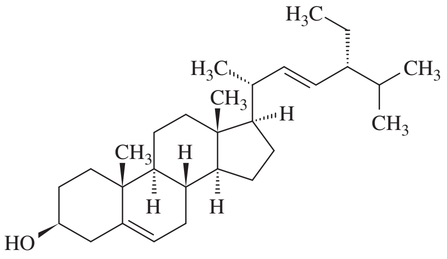
3-121, Δ^5-avenasterol



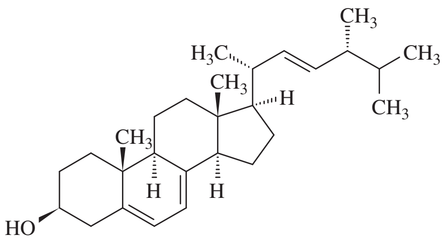
3-122, clerosterol



3-123, fucosterol



3-124, spinasterol

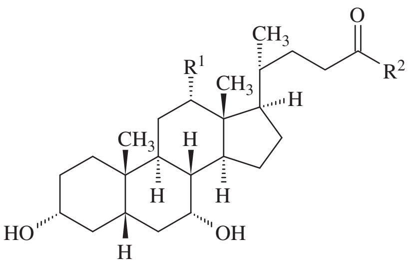


3-125, ergosterol

3.7.4.2 Biochemistry, physiology and nutrition

Steroids are synthesised in organisms via complex mechanisms from isoprene units, isopentenyl diphosphate and dimethylallyl diphosphate, which first yield geranyl diphosphate. Reaction with another molecule of isopentenyl diphosphate gives an important intermediate, farnesyl diphosphate (Figure 3.10). Two molecules of farnesyl phosphate give rise to triterpenic hydrocarbon squalene (**3-81**), which in the body of animals yields triterpenic alcohol lanosterol (**3-92**) and the triterpenic alcohol cycloartenol (**3-93**) in plants.

Plants synthesise a number of steroid substances from cycloartenol, including 4,4-dimethylsterols, 4-methylsterols and demethylsterols. Cycloartenol is a precursor of many other steroids that are aglycons of saponins, of steroidal glycoalkaloids and of other compounds. Lanosterol in animals is a precursor for the biosynthesis of the most important zoosterol cholesterol (**3-114**). An intermediate in the biosynthesis of cholesterol is 7-dehydrocholesterol, which is a precursor of vitamin D_3 (see Section 5.3.1). Cholesterol in the body is used for the biosynthesis of steroid hormones[17] and bile acids[18] (**3-126**).



3-126, bile acids and their conjugates

chenodeoxycholic acid, $R^1 = H$, $R^2 = OH$
glycochenodeoxycholic acid, $R^1 = H$, $R^2 = NHCH_2COOH$
taurodeoxycholic acid, $R^1 = H$, $R^2 = NHCH_2CH_2SO_3H$
cholic acid, $R^1 = OH$, $R^2 = OH$
glycocholic acid, $R^1 = OH$, $R^2 = NHCH_2COOH$
taurocholic acid, $R^1 = OH$, $R^2 = NHCH_2\ CH_2SO_3H$

The main bile acids are cholic acid and chenodeoxycholic acid (**3-126**). These acids are excreted from the liver as glycine salts or taurine conjugates (see Section 5.15) into the gallbladder and then into the small intestine. In the duodenum, bile acids act as emulsifiers in the absorption and digestion of fats and fat soluble vitamins.

Sterols are essential components of lipoproteins and lipid membranes in animals. They are particularly important in nerve tissues and in the transport of lipids (fatty acids), which are bound in lipoproteins. In humans, dietary cholesterol intake is lower than the daily requirement, therefore the body synthesises the majority of cholesterol that is needed (a larger intake in the diet decreases the amount synthesised by the body). Cholesterol in the diet is easily absorbed, but problems may occur during transport of cholesterol from the intestinal wall during lymph and blood circulation. Excessive cholesterol transport in low-density lipoproteins (LDL) may cause health problems. It is therefore recommended that the intake of dietary cholesterol should not exceed 300 mg per day.

[17]Steroid hormones are divided into five groups: progestins, glucocorticoids, mineralocorticoids, androgens and oestrogens (see Section 10.3.2.5.1). They mediate many important functions in living organisms.

[18]The recycling system allows bile acids to re-enter the bloodstream and return to the liver for re-use. They are partially (some 1000 mg per day) metabolised in the colon by microorganisms, which cleave the peptide bond of conjugates and bile acids and are converted into deoxycholic ($R^1 = OH$, $R^2 = OH$) and lithocholic ($R^1 = H$, $R^2 = OH$) acids (the hydroxyl group at C-7 is replaced by H atom in both compounds), which is excreted in the faeces from the body. This is the only way of removing cholesterol indirectly from the body. The amount of excluded bile acids may be increased due to their binding to the soluble fibre, such as pectin, fructooligosaccharides or arabinoxylans.

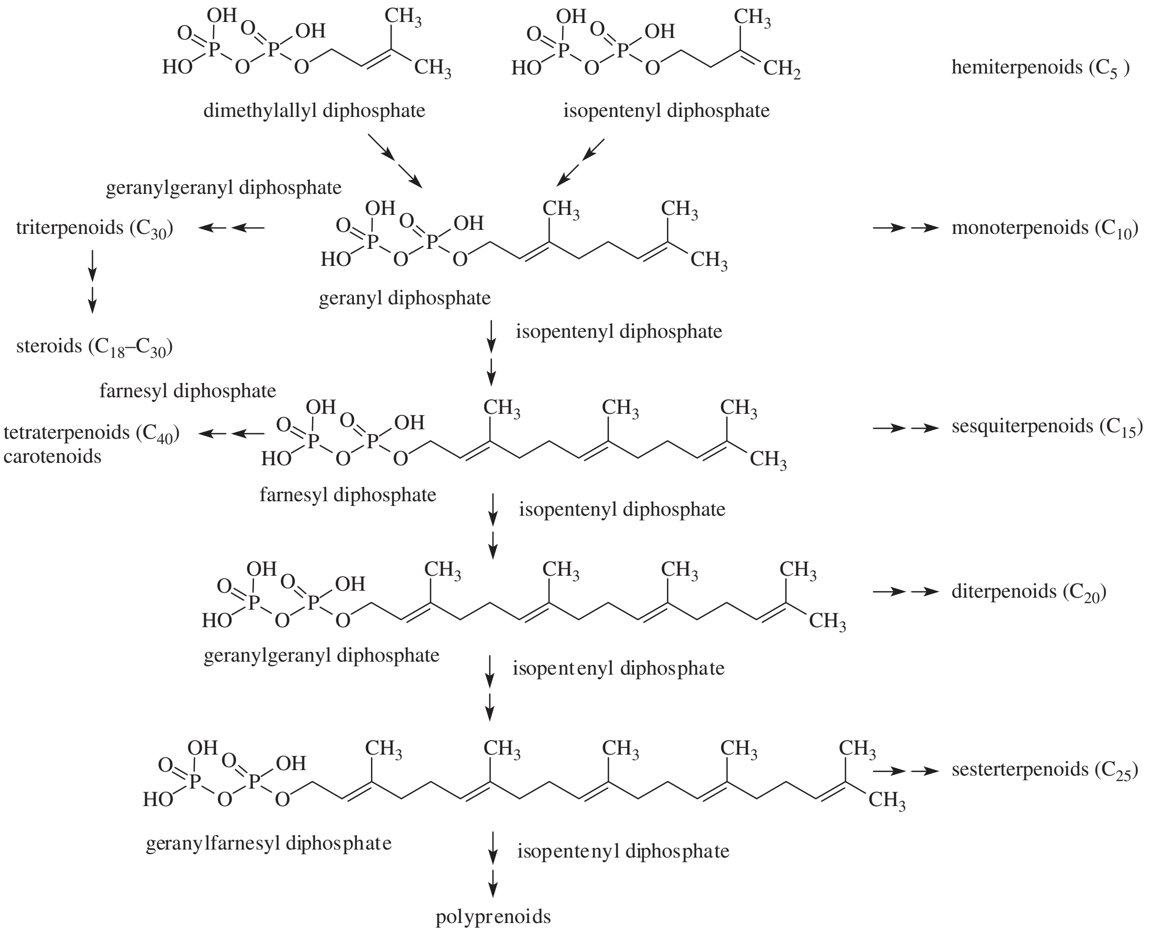


Figure 3.10 Biosynthesis of steroids and other terpenoids.

However, its intake is often more than twice as high in developed countries, therefore reducing dietary fat is recommended to reduce the intake of dietary cholesterol.

Dietary phytosterols have some influence on the biosynthesis of cholesterol in the body, although they are not used for making membranes and in the body they break down. To reduce the amount of cholesterol in blood plasma, the Recommended Daily Intake of phytosterols is about 250 mg. There are now margarines containing about 10% phytosterols (in the form of phytostanol esters derived from tall oil or in the form of sterol esters isolated from soybean oil). The importance of triterpene alcohols in the diet is not known.

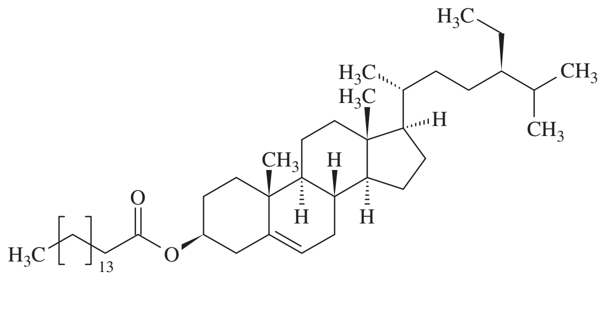
3.7.4.3 Occurrence

Steroids in food of animal and vegetable origin appear as:

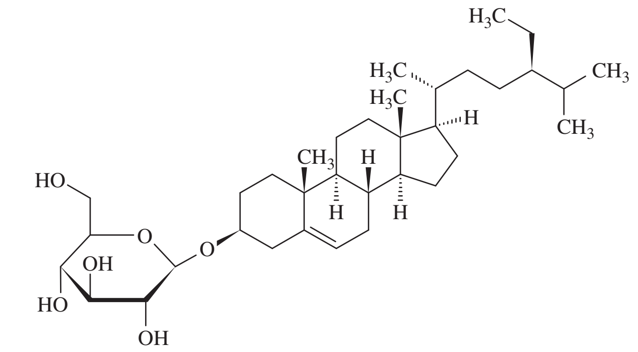
- **free compounds**
- **esters** with higher fatty acids and cinnamic acids
- **glycosides** (common sugars are D-glucose and D-mannose)
- **glycoside esters** of higher fatty acids (called acylsterylglycosides).

Examples of these compounds are β-sitosteryl palmitate (**3-127**), β-D-glucopyranosyl-β-sitosterol (**3-128**) and its ester with palmitic acid (**3-129**). In sunflower oil, for example, the amount of steroids is 0.32%, 0.07% of steroids occur as esters, 0.03% as glycosides and the rest are free sterols. Fatty acids bound in sterol esters are mainly palmitic, stearic, oleic, linoleic and linolenic acids, but the fatty acid composition of sterol esters may not match the composition of fatty acids in triacylglycerols.

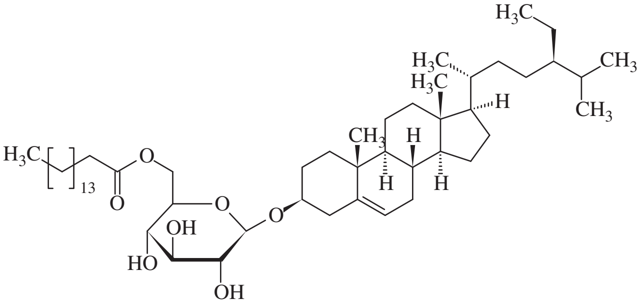
Ferulic acid esters of phytosterols (steryl ferulates) have been identified in rice, wheat, rye, triticale and barley. The highest amounts of ferulated phytosterols occur in rice. They are also



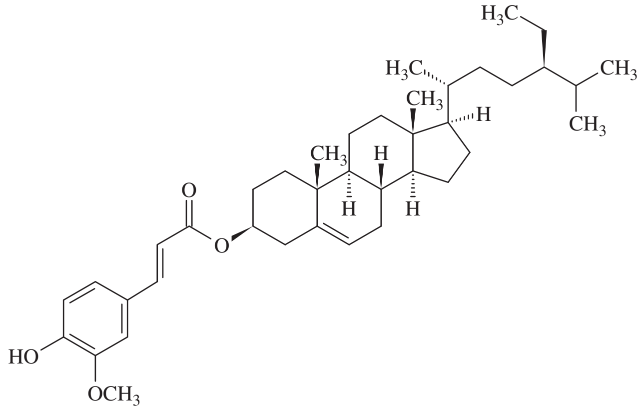
3-127, β-sitosteryl palmitate



3-128, β-D-glucopyranosyl-β-sitosterol



3-129, 6-*O*-palmitoyl-β-D-glucopyranosyl-β-sitosterol



3-130, β-sitosteryl ferulate

found in rice bran oil, but are removed during refining. Their mixture is called **γ-oryzanol**. Ferulic acid esters of 4,4′-dimethylsterols (cycloartenyl ferulate and 24-methylenecycloartanyl ferulate) and of 4-demethylsterols (campesteryl ferulate, campestanyl ferulate, stigmasteryl ferulate and β-sitosteryl ferulate, **3-130**) have been identified as major components of γ-oryzanol. The γ-oryzanol content in brown rice of European origin ranges from 260 to 630 mg/kg. The main components in wheat and rye grains are campestanyl ferulate and sitostanyl ferulate, followed by campesteryl ferulate and β-sitosteryl ferulate. Steryl ferulates are valued for their antioxidant properties.

3.7.4.3.1 Animal fats

In practice, cholesterol is mainly found in animal fats and in human tissues (Table 3.39). In lower animals other sterols, known collectively as zoosterols may also be present.

Cholesterol and its esters are present in all membranes and in blood lipids, but particularly rich sources are nervous tissues, especially the brain. Therefore, the cerebellum would be a food with the highest cholesterol content. Also, egg yolks contain high amounts of cholesterol. Other important sources include meat, milk and cheeses, but also animal fats, lard and butter to a greater extent. Together with cholesterol and other steroids, wool fat contains 4,4-dimethylsterol, known as lanosterol, as the main steroid.

3.7.4.3.2 Vegetable oils

Several steroid compounds are usually present in plants and other organisms, but the major steroids are phytosterols. Those found in higher plants (β-sitosterol being the most predominat) and some other organisms is shown in Table 3.40.

The composition of major sterols found in several vegetable oils is shown in Table 3.41. The total content of phytosterols in vegetable oils is given in Table 3.42. Usually, vegetable oils contain a mixture of phytosterols and related compounds, which are characteristic of a particular oil. Cholesterol is also a phytosterol as it is found in many vegetable oils, but is usually present at very low levels that have no practical importance in the nutritional balance. Vegetable oils also contain numerous 4,4-dimethylsterols and 4-methylsterols in small quantities. The contents of these compounds and other steroids in maize oil are given in Table 3.43 as an example.

3.7.4.3.3 Other lipids

In yeast and fungi (molds and higher fungi) the main sterol is ergosterol (**3-125**), which belongs, along with related compounds (precursors and their metabolites), to the C_{28} mycosterols. Ergosterol usually represents 60–70% of the total sterols present. Other sterols are primarily derived from ergosterol, such as ergosta-5-en-3β-ol, ergosta-7-en-3β-ol, ergosta-5,7-dien-3β-ol (dihydroergosterol), ergosta-5,22-dien-3β-ol, ergosta-7,22-dien-3β-ol, ergosta-5,24(28)-dien-3β-ol, ergosta-7,24(28)-dien-3β-ol and ergosta-5,7,9(11),22-tetraen-3β-ol.

Other minor steroids are C_{27} sterols, such as cholesterol and desmosterol, and C_{29} sterols, for example β-sitosterol, stigmasterol and other sterols. Sterols are found in fungi as free compounds, as fatty acid esters (mainly esters of linoleic, oleic and palmitic acids) and as glycosides in which the sugar component is D-glucose.

3.7.4.4 Properties

Sterols are solid crystalline substances with high melting points, which are insoluble in water, poorly soluble in alcohol, but soluble in non-polar solvents. Pure sterols are relatively stable in air during storage, but they are easily oxidised in solutions. Catalytic hydrogenation of oils transforms sterols into the corresponding saturated compounds.

Table 3.39 Cholesterol content in foods of animal origin.

Food		Content (mg/kg edible portion)
Meat, meat products, poultry, fish	Beef	590–670
	Mutton	700–720
	Veal	650–700
	Pork	600–760
	Sausages	470–1150
	Poultry	650–900
	Fish	420–1500
Milk and dairy products	Milk	120–140
	Yoghurt	40–100
	Cream	190–1050
	Cheesses	290–1050
	Curd	50–130
Fats	Beef tallow	1000
	Butter	2400
	Pork lard	940
Eggs	Egg yolk	8400–13 100
	Egg white	0
	Eggs (whole)	2000–3540
Pastry[a]	Mayonnaise	1100
	Pastry (various products)	150–2800

[a] Almost exclusively from eggs and animal fats.

Table 3.40 Main sterols in plants and other organisms.

Organism	Main sterols	Organism	Main sterols
Bacteria	Cholesterol, β-sitosterol	Brown algae	Fucosterol, 24-methylenecholesterol
Lower fungi	Ergosterol	Red algae	Cholesterol, desmosterol
Higher fungi	Ergosterol	Mosses	β-Sitosterol
Lichens	Ergosterol, lichesterol, poriferasterol	Ferns	β-Sitosterol
Green algae	Various Δ^5-, Δ^7- and $\Delta^{5,7}$-sterols	Higher plants	β-Sitosterol, other Δ^5- and Δ^7-sterols

3.7.4.5 Use

Sterols are a regular part of the diet. The most common sterol is cholesterol, which is used in the cosmetics and pharmaceutical industries as an emulsifier and as a starting material for the synthesis of various biologically active derivatives, such as vitamin D_3 or steroid hormones. During deodorising of vegetable oils, a mixture of phytosterols can be obtained, which can become a viable addition to a variety of dietary products such as margarines (see Section 3.7.4.2).

3.7.5 Lipophylic vitamins

Edible vegetable oils regularly contain varying amounts of tocopherols (vitamin E) or, rarely, their esters. Synthetic all-rac-α-tocopheryl acetate (D,L-α-tocopheryl acetate) is often added to oils and other products as a food additive. The fat products often contain synthetic retinyl acetate (vitamin A), which is more stable than retinol. Emulsified fats sometimes contain vitamin D. Animal fats contain only small amounts of lipophilic vitamins, except for

Table 3.41 Composition of sterols in vegetable oils (expressed as % of total sterols).

Sterol	Rapeseed oil	Soybean oil	Sunflower oil	Palm oil
Cholesterol	0.5-1.3	0.6-1.4	<0.5	2.6-6.7
Brassicasterol	5.0-13.0	0.0-0.3	<0.1	0
Δ^5-Campesterol	24.7-38.6	15.8-24.2	8-12	18.7-27.5
Stigmasterol	0.0-0.7	15.9-19.1	7-11	8.5-13.9
β-Sitosterol	45.1-57.9	51.7-57.6	50-62	50.2-62.1
Δ^5-Avenasterol	3.1-6.6	1.9-3.7	1.5-7	0-2.8
Δ^7-Stigmasterol	0.0-1.3	1.4-5.2	20	0.2-2.4
Δ^7-Avenasterol	0.0-0.8	1.0-4.6	3-6.5	0-5.1
Δ^7-Campesterol	-	-	2-3	-
Clerosterol	-	-	0.7-1.0	-

Table 3.42 Total content of sterols in some vegetable oils.

Oil	Phytosterols content (mg/kg)	Oil	Phytosterols content (mg/kg)
Olive	>1000	Sunflower	2437-4545
Peanut	901-2854	Rapeseed	4824-11 276
Cotton	2690-6425	Rice	10 550
Maize germ	7950-22 150	Palm	376-627
Wheat germ	5500	Palm kernel	792-1406
Sesame	4 01-18 957	Almond	2660
Safflower	2095-2647	Hazelnut	1200
Soybean	1837-4089	Hempen	3700

fish oils, which are sometimes very rich in vitamins A (see Section 5.2.5.3) and D (see Section 5.3.5.1).

3.7.6 Lipophilic pigments

Plant lipids contain various natural dyes, particularly carotenoids (see Section 9.9.2.3.3) and chlorophylls (see Section 9.2.2). Of the animal fats, only butter has an important natural carotenoid pigment content. Carotenoid pigments are added to margarines as additives (see Section 11.4.1.5).

3.7.7 Natural antioxidants

In addition to tocopherols, vegetable oils, especially olive, sesame and soybean oils, contain natural antioxidants that are described elsewhere (see Section 11.2.2.4).

Table 3.43 Major steroids in maize oil.

Steroids	Composition (%)	Steroids	Composition (%)
4,4-Dimethylsterols		***4-Demethylsterols***	
Cycloartenol	0.6	Cholesterol	traces
24-Methylenecycloartanol	1.4	24-Methylenecholesterol	1.3
Cyclosadol	traces	Stigmasterol	7.3
4-Methylsterols		Isofucosterol	9.0
Obtusifoliol	1.2	Campesterol	9.3
24-Methylenelophenol	1.0	24-Epicampesterol	13.9
Citrastadienol	0.5	β-Sitosterol	52,5

3.8 Reactions

3.8.1 Reactions of fatty acids

Chemical reactions of fatty acids involve the carboxyl group, as well as the hydrocarbon residue of the molecule, particularly the double bonds and neighbouring methylene groups. The reactions of the hydrocarbon chain are common to free fatty acids and to their esters.

3.8.1.1 Formation of salts

Although fatty acids are relatively weak acids, they readily form salts with metal cations or organic bases, most easily with alkali metal cations and with ammonia. These compounds are called **soaps**.

Soaps of alkali metals are called tensides. Sodium soaps of unsaturated fatty acids and medium-chain saturated fatty acids are very soluble in water (this corresponds to a high critical micellar concentration), but soaps of saturated acids with long chains ($>C_{14}$) dissolve better if the temperature is raised (this corresponds to a very low critical micellar concentration). Technologically this is utilised specifically in the formation of sodium soaps in alkaline neutralisation (refining) of vegetable oils, when free fatty acids are soluble in oil, but their soaps are soluble in water. This reaction occurs at the water/oil interface in an emulsion. Sodium salts of fatty acids are important for the production of toilet soaps, detergents and cleaning agents. Potassium soaps are more alkaline and have a significantly higher detergent effect, but are now limited in their production.

Soaps of divalent cations and aluminium soaps are called **metal soaps**. They have a hydrophobic character and therefore are of great significance in non-food applications in industry and construction. They are seldom found in foods. Calcium and magnesium soaps commonly arise from alkaline soaps in hard water, but they are also natural components of foods at low levels. The concentrations at which soaps occur naturally have no significant effect on the sensory and nutritional value of a food or its functional properties. However, higher concentrations of soaps may lower the acidity and negatively affect some functional properties of the food and cause diarrhoea. Calcium soaps may be used as a source of calcium in the diet of dairy cows.

Soaps only form true solutions in water at a high dilution. Concentrated solutions of soaps, after exceeding the so-called critical micellar concentration, form micelles, which are agglomerations of many molecules where the polar groups are directed to the surface and the hydrocarbon chains into the interior of micelles. Solutions of alkali soaps hydrolyse in water and thus produce an alkaline solution. Salts of divalent cations, mainly of calcium and magnesium, are practically insoluble in water and in organic solvents they only dissolve with difficulty. These salts are mixtures of varying compositions and with different ratios of cations and acyls. Soaps of fatty acids and other acidic components, such as phosphoric acid derivatives, can also occur in foods.

3.8.1.2 Esterification reactions

One of the main reactions of the carboxyl group of fatty acids is esterification. This is an ionic reaction, which is usually catalysed by acids, that proceeds similarly to the esterification of other organic carboxylic acids. Esterification of higher fatty acid usually takes place at high temperatures (autocatalysis) or under catalysis of strong acids (Figure 3.11). In fatty acids in foods the process is often catalysed by lipases. Esterification or interesterification of fatty acids, which is used in specific instances, is catalysed by microbial lipases. This is discussed again in the section dealing with reactions of triacylglycerols, because it is of greater significance in their conversion.

In food technology, esterification is usually employed in the production of monoacylglycerol emulsifiers, obtained by esterification of glycerol with fatty acids. The main reaction products are mono- and diacylglycerols. Interesterification (glycerolysis) of fat is usually a more convenient reaction. Similarly, various other emulsifiers that are used as food additives (see Section 11.5.2) are produced from glycols, glycerol, hydroxy acids and sugars. Esterification is also utilised in the manufacture of particular dietary fats (triacylglycerols) with medium chain fatty acids (caproic and capric). A wide range of esters that act as wax analogues, such as 2-propyltetradecanoate (2-propylmyristate), are manufactured by the pharmaceutical and cosmetics industries.

The carboxyl group can react also with the hydroxyl group of hydroxy acids that are produced either during the oxidation of fats or occur naturally in the fats. These reactions (which are in fact polycondensations) yield products called estolides (Figure 3.12).

3.8.1.3 Formation of amides

Naturally occurring fatty acid amides are described in Section 3.6.1. In non-food uses of lipids, fatty amides are produced synthetically in industry for use as ingredients of detergents, lubricants, inks and many other products. Ammonium salts of fatty acids are gradually dehydrated to amides and aliphatic nitriles that are used for the production of fatty primary amines, which are then used as reactants for the synthesis of cationic and amphoteric surfactants.

Amides of fatty acids are also formed from fatty acids during thermal treatments by reaction with ammonium salts, amines or amino acids (see Section 8.2.10.1.3).

3.8.1.4 Isomerisation reactions of unsaturated acids

Double bonds of unsaturated fatty acids can isomerise so that they change their steric configuration (*cis–trans* isomerisation, also

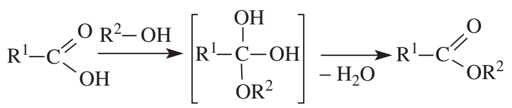


Figure 3.11 Esterification of fatty acids.

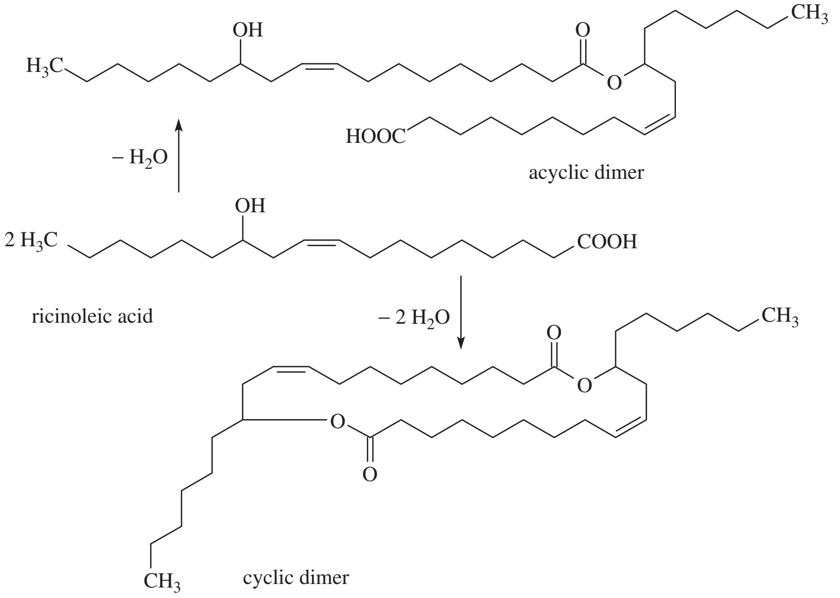


Figure 3.12 Formation of estolides from ricinoleic acid.

known as geometrical isomerisation) or so that the double bond shifts in the hydrocarbon chain (positional isomerisation).

3.8.1.4.1 Geometrical isomerisation

Double bonds of unsaturated fatty acids in natural lipids are almost exclusively in the *cis* configuration, although *trans* double bonds are thermodynamically more stable. Under equilibrium conditions, the ratio of *cis* to *trans* double bonds is approximately 30:70. Therefore, under appropriate conditions (if free radicals are temporarily created and electrons can move to the carbon with an sp^2 hybridisation) an interconversion between *cis* and *trans* isomers occurs. This happens, for example, during heating, autoxidation and hydrogenation.

Thermally induced geometrical isomerisation is a non-equilibrium reaction, which means that the isomerisation proceeds until exhaustion of the *cis* double bonds. The degree of isomerisation achieved is thus a function of temperature and time of heating. This isomerisation occurs in particular during deodorising and physical refining of oils and fats. Monoenoic fatty acids (such as oleic acid) require heating to temperatures around 270 °C at which *cis–trans* isomerisation of the allylic system of one double bond proceeds at a reasonable rate. Pentadienoic systems of isolated double bonds in dienoic acids, which have two double bonds separated by methylene group, are far less stable.

The splitting off of a hydrogen radical from the methylene group adjacent to the double bonds creates a free radical transformed into the mesomeric form (Figure 3.13 and Figure 3.14). The

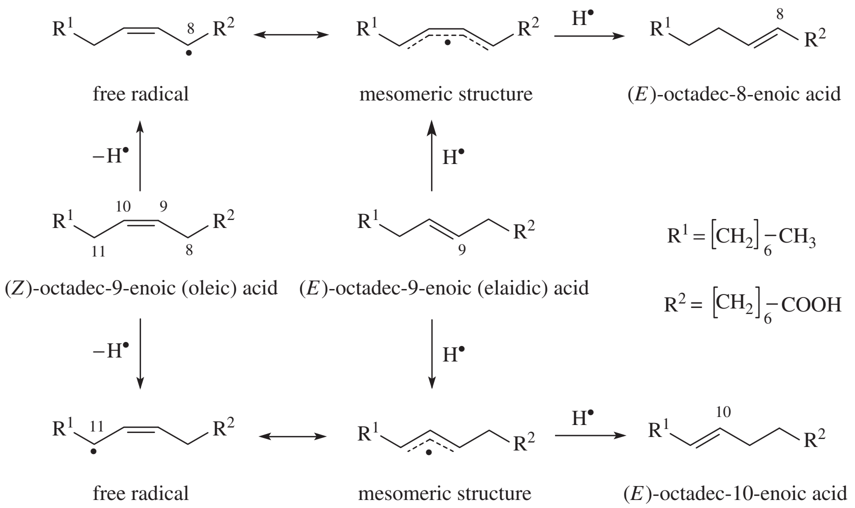


Figure 3.13 Isomerisation of monoenoic fatty acids.

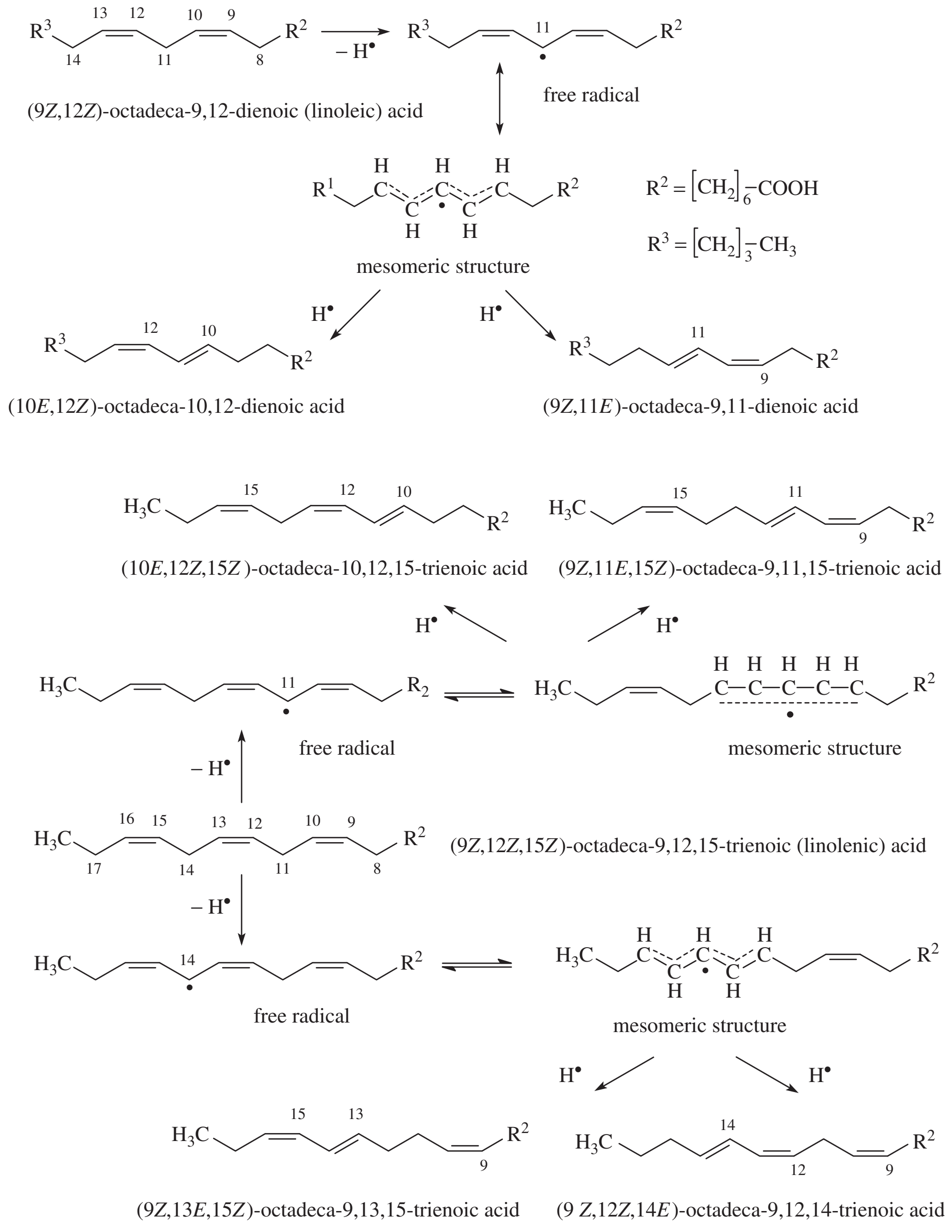


Figure 3.14 Isomerisation of polyenoic fatty acids.

reaction of the mesomeric form with a hydrogen radical forms the most stable system possible, which is usually a system with one or two *trans* double bonds. Therefore, *cis–trans* isomers and *trans–trans* isomers of linoleic acid form at a significant rate at temperatures 240 °C and higher. Therefore, deodorised edible oils always contain small amounts of isomerised dienoic acids. Acids with three double bonds have an even greater tendency to isomerise, because each acyl contains two labile methylene groups. Linoleic acid yields *cis–trans*, *trans–cis* and *trans–trans* isomers. Linolenic acid mainly produces *cis–cis–trans*, *trans–cis–cis*, *cis–trans–cis* and *trans–cis–trans* isomers at a significant rate at temperatures of 210–220 °C and higher. Oils with a higher content of trienoic, tetraenoic, pentaenoic and hexaenoic acids are very easily isomerised at temperatures above 200 °C, which means that in practice these oils cannot be deodorised.

In parallel with saturation of the double bonds during the catalytic hydrogenation of oils, geometrical isomerisation of fatty acids via intermediate free radicals also occurs. Isomerisation using a nickel catalyst proceeds to equilibrium; therefore, partially hydrogenated fats can contain 50–60% of *trans* isomers of fatty acids, especially of octadecenoic acids. The main isomer is usually elaidic acid. Similarly, *trans* isomers of octadecenoic acid are formed by biohydrogenation in the composite stomach of ruminants where the main *trans* isomer is vaccenic acid. The process, in this case, is far from equilibrium so the *trans* isomers content in the milk fat of ruminants is about 3–6%.

The double bond also isomerises during oxidation, which begins with the formation of a free radical at the position adjacent to the double bond. In this case, the amount of *trans* isomers is proportional to the amount of formed hydroperoxides.

3.8.1.4.2 Positional isomerisation

In unsaturated fatty acids, positional isomerisation is also possible, when the double bond shifts by one carbon atom either away from the carboxyl or towards the carboxyl. Positional isomerisation is usually associated with *cis–trans* isomerisation and takes place under similar conditions to the *cis–trans* isomerisation, on heating to temperatures higher than about 240 °C. Isolated double bonds are relatively stable, but the pentadiene systems easily isomerise to conjugated systems. Two conjugated isomers are formed from linoleic acid, whereas linolenic acid yields four conjugated isomers (Figure 3.14).

In deodorised oils, where the oil temperature reaches 220–260 °C for several tens of minutes, conjugated dienes are always present in small amounts. The isomerisation is catalysed by bleaching clays and other materials with an active surface. Conjugated triene systems do not accumulate in oils, as they are quickly cyclised or polymerised.

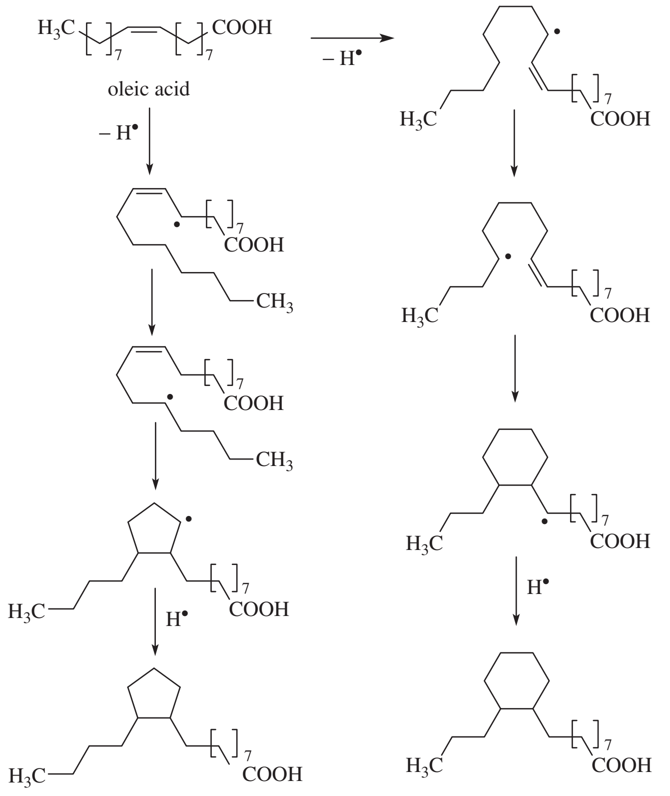
Conjugated unsaturated fatty acids also form at the beginning of hydrogenation and during autoxidation of unsaturated fatty acids. In these processes, even isolated double bonds isomerise. The partially hydrogenated oils, which originally contained linoleic and linolenic acids, in addition to isomers with double bonds at positions C-9, C-12 and C-15, thus also contain other isomers of octadecenoic acids with double bonds in positions C-5 to C-16.

Geometric and positional isomers arising from biohydrogenation of unsaturated fatty acids in the rumen of ruminants become components of lipids of milk and meat. Linoleic acid yields a mixture of positional and geometric isomers called conjugated linoleic acid. The main constituents of this mixture are (9*Z*,11*E*)- and (9*E*,11*Z*)-octadecadienoic acids. The first acid represents about 90% of conjugated acids of cows' milk and more than 75% of the conjugated acids of tallow. Also formed in small amounts are (8*E*,10*Z*), (9*Z*,11*Z*)-, (9*E*,11*E*)-, (10*Z*,12*Z*)- and (10*E*,12*E*)-octadecadienoic acids and so on. Conjugated linoleic acid has some beneficial effects, for example anticarcinogenic, antiatherogenic, antidiabetic and immunomodulatory.

3.8.1.5 Cyclisation of unsaturated acids

The unsaturated fatty acids are also transformed, via the radicals formed during heating to higher temperatures (e.g. during frying), into cyclic fatty acids with five- and six-membered rings. Cyclic acids derived from oleic acids are saturated compounds. The products formed from linoleic acid have one double bond. These products include cyclopentene acids and cyclopentane and cyclohexane acids with one *cis* double bond in the side chain. Linolenic acid forms products with two double bonds, such as cyclopentene and cyclohexene acids with one *cis* double bond in the side chain.

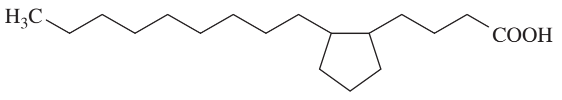
After deep frying, oils contain cyclic fatty acids bound in triacylglycerols (free acids evaporate during frying) at a concentration



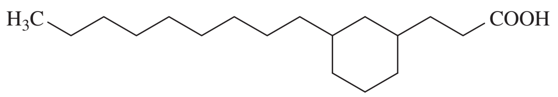
9-(2-butylcyclopentyl)nonanoic acid 9-(2-propylcyclohexyl)nonanoic acid

Figure 3.15 Mechanism of cyclic fatty acids formation.

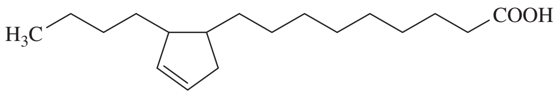
of 0.1–7 g/kg. There are approximately twice as many acids with five-membered rings as with six-membered ring. The mechanism for the formation of cyclic fatty acids from oleic acid is given in Figure 3.15. The structures of other products derived from oleic acid are represented in formulae **3-131** and **3-132**, two of the numerous products of linoleic acid cyclisation are given in formulae **3-133** and **3-134**, whilst formulae **3-135** and **3-136** show two of the many cyclisation products of linolenic acids.



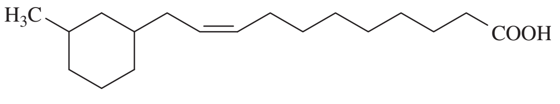
3-131, 4-(2-nonylcyclopentyl)butanoic acid



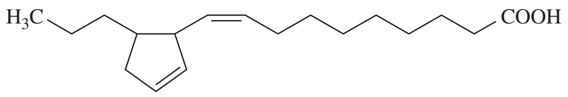
3-132, 3-(3-nonylcyclohexyl)propanoic acid



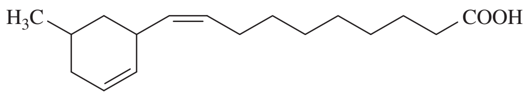
3-133, 9-(2-butylcyclopent-3-en-1-yl)nonanoic acid



3-134, (*Z*)-11-(3-methylcyclohexyl)undec-9-enoic acid



3-135, (*Z*)-10-(2-propylcyclopent-4-en-1-yl)dec-9-enoic acid



3-136, (*Z*)-10-(3-methylcyclohex-5-en-1-yl)dec-9-enoic acid

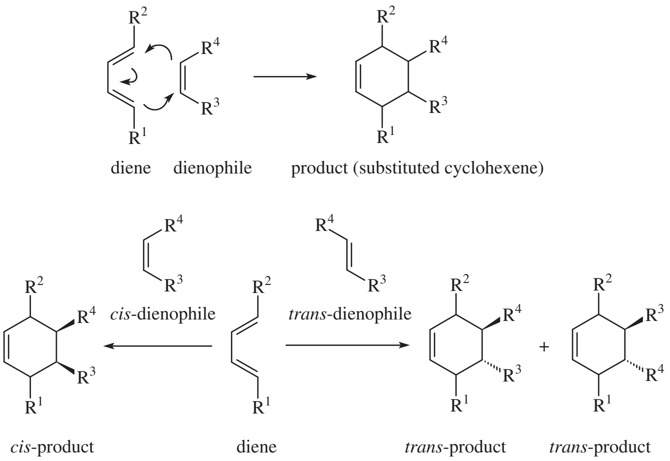


Figure 3.16 Diels–Alder mechanism of polymerisation of polyenoic fatty acids (R^1, R^3 = residues of hydrocarbon chains, R^2, R^4 = residues of chains with carboxylic groups).

3.8.1.6 Polymerisation of unsaturated acids

Polymerisation refers to processes in which the overall composition of a compound does not substantially alter, but the molecular weight increases by a multiple of the weight of the monomer. Polymers usually arise when a system can create free radicals. Therefore, polymerisation reactions usually accompany isomerisation reactions and the formation of cyclic fatty acids under extreme heating. In the fully refined (and thus also deodorised) edible oils, polymers represent several tenths of a percent, but their content increases during heating. Oils with a polymer content of more than 10% are not recommended for use.

Under a low partial pressure of oxygen and at higher temperatures (such as during frying), cyclic and linear polymers are formed, in which the original fatty acids are bound together mainly by C–C bonds. When an adequate supply of air is present (in an environment with a high partial pressure of oxygen) and at lower temperatures, the oxidation of polyenoic fatty acids produces a large number of free radicals containing oxygen. In addition to cyclic and linear polymers of the type C–C, their recombination yields linear polymers with ether bonds (C–O–C) and linear polymers with peroxide bonds (C–O–O–C). Therefore, oxidation is always accompanied by polymerisation, especially in more advanced stages of the reaction. Such a polymerisation is called **oxypolymerisation**. Oxypolymerisation is associated with secondary polymeric products of autoxidation of fatty acids. Under typical conditions encountered during food processing, monoenoic fatty acids barely polymerise and dienoic fatty acids usually only form dimers. Trienoic fatty acids, however, polymerise very readily, and can also form higher oligomers.

Cyclic dimers, which are actually substituted cyclohexene derivatives, arise during polymerisation by the Diels–Alder reaction. This reaction takes place via a cyclic transition state and is therefore also referrd to as cycloaddition. In this reaction, the so-called dienophile, a substituted alkene (olefin), which is a monoenoic fatty acid, reacts with a conjugated *cis,cis*-dienoic fatty acid (Figure 3.16).

If the dienophile has two different substituents, various stereoisomers may be formed. If the substituents in the dienophile are in the *cis* position, the reaction product (substituted cyclohexene) also has substituents in the *cis* position and vice versa.

The reaction can take place intermolecularly, between two fatty acids bound in two different triacylglycerol molecules, or intramolecularly, within a single triacylglycerol fatty acid. An example of a cyclic dimer of the cyclohexene type is the reaction of linoleic acid with 10,12-octadecadienoic acid (Figure 3.17). An example of a cyclohexene structure resulting from the intramolecular reaction of two unsaturated fatty acids bound in a triacylglycerol molecule is shown in Figure 3.18. Cyclic dimers of the cyclohexene type containing an additional double bond and also a system of conjugated double bonds may react further to form tricyclic dimers (Figure 3.19). If the trienoic fatty acids forming a dimer still contain

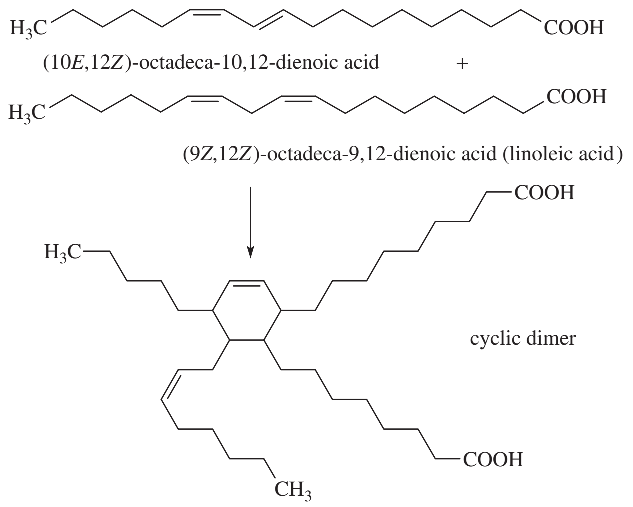


Figure 3.17 Dimerisation of dienoic fatty acids to cyclohexene derivatives.

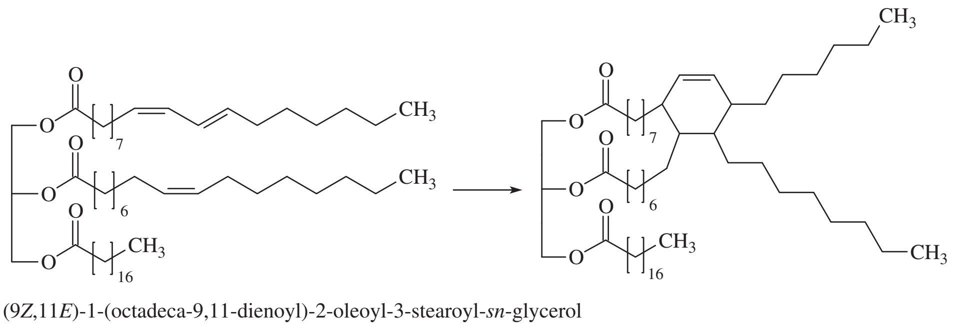


Figure 3.18 Structure of cyclic dimers in a triacylglycerol molecule.

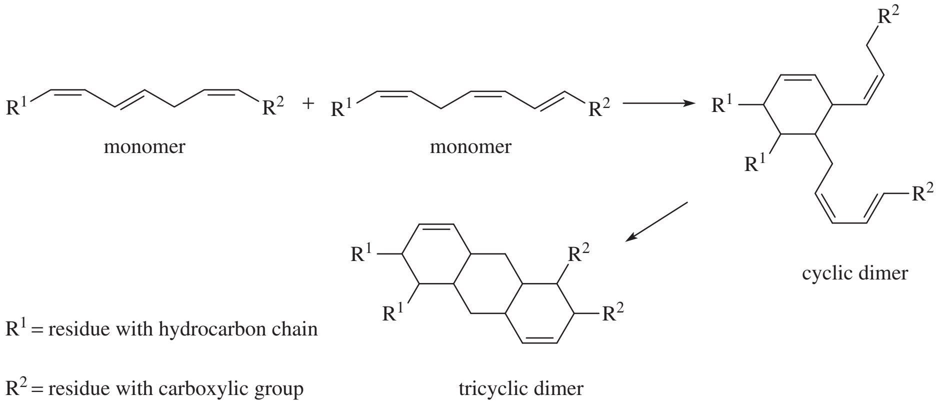


Figure 3.19 Formation of condensed bicyclic dimers.

a system of two conjugated double bonds in the molecule, there is the possibility of further reaction to form a trimer (Figure 3.20).

In addition to the cyclic dimers of the cyclohexene type, some cyclic cyclopentane C–C dimers and acyclic C–C dimers may also be formed. The mechanism of their formation (Figure 3.21) is demonstrated for reactions of one of the four main free radicals generated from oleic acid. An acyclic dimer forms by recombination of two radicals (reaction A in Figure 3.21), but also by addition of a radical to the double bond of another radical (reactions B and C in Figure 3.21) and by a reaction (termination) with a hydrogen radical (reaction D in Figure 3.21). The formation of bicyclic derivatives is illustrated in Figure 3.22.

In practice, mixed linear polymers in fats arise from radicals of different fatty acids. Free radicals can also be formed by splitting

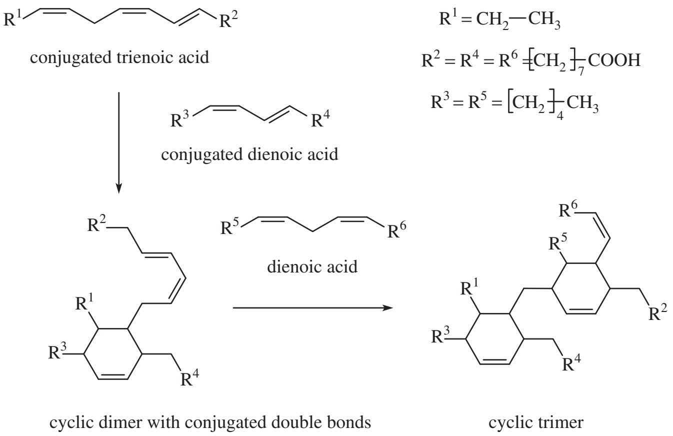


Figure 3.20 Formation of oligomers from trienoic fatty acids.

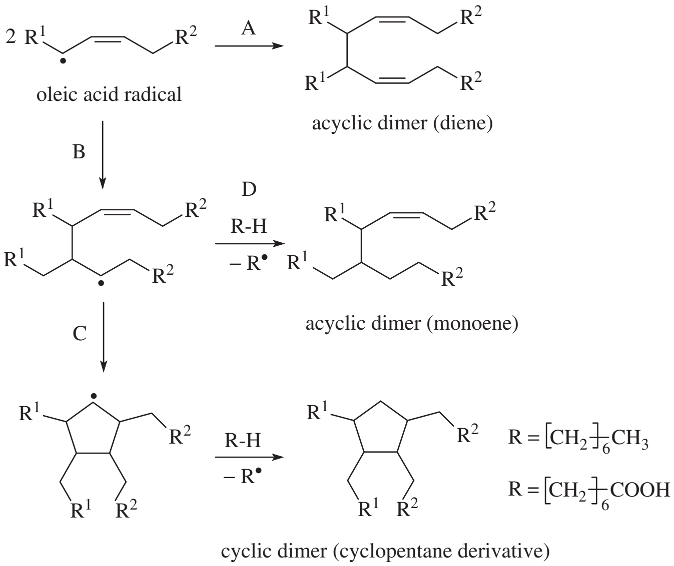


Figure 3.21 Formation of dimers from free radicals of oleic acid.

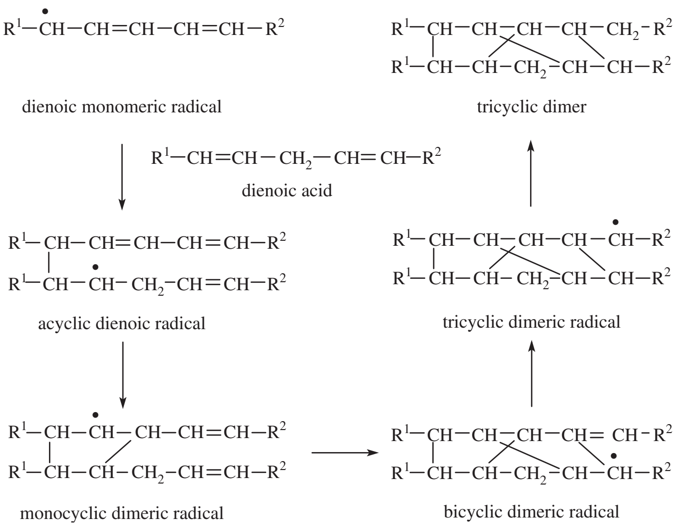


Figure 3.22 Formation of oligocyclic dimers in heated dienoic fatty acids.

the chain around the double bond. They therefore have a smaller number of carbon atoms, and their combinations with free radicals of fatty acid that have not split yield a dimer with the number of carbon atoms being between a monomer and a dimer. In oils used for frying, cyclohexene and cyclopentane structures, bicyclic derivatives, acyclic dienes and other products have been identified. Polymerisation mainly occurs in fats exposed to higher temperatures for a long time, for example in frying oils. It manifests itself through colour changes and deterioration of fat functional properties, mainly by foaming and increased viscosity.

For example, oleochemistry produces specific so-called dimeric fatty acids for the production of adhesives.

3.8.1.7 Hydrogenation reaction of unsaturated acids

Hydrogenation of double bonds of fatty acids, technically this is commonly known as hardening of oils and fats, is a chemical modification of oils and fats in the double bonds of unsaturated fatty acids. The common confusion between the terms 'hydrogenation' and 'hardening' reflects the fact that the primary objective when introducing this technology was to change the consistency of liquid oils and fats into viscoplastic substances of a required consistency. Hydrogenation was an important technological development in the fat industry. Owing to the growth of human population in the 19th and 20th centuries, the traditional animal fats, lard and butter, that were suitable for baking, for addition to dough, for use as spreadable fats and other purposes, became scarce. At the same time, there was a relative abundance of fish oils and whale blubber[19] in the world, but these fats were not suitable for direct use because of their adverse physical and organoleptic properties. Therefore, suitable procedures were sought to produce solid fats (correctly known as viscoplastic thixotropic substances) from liquid oils. The best procedure seemed to be the industrial hydrogenation of double bonds, which was introduced in 1902–1910. Paradoxically, in Europe, specifically oils and fats of animal origin were originally hydrogenated, while in the United States soybean and cottonseed oils began to be hydrogenated gradually before World War II. In Europe, vegetable oils began increasingly hydrogenated in the 50th when the whaling era ended.

3.8.1.7.1 Hydrogenation of double bonds

Oils can be either partially or fully hydrogenated through the hydrogenation process. Partially hydrogenated oils, such as those found in salad dressings, produce hardened – but not solid – fats. Fully hydrogenated oils, such as shortenings, are solid fats. Food items that contain hydrogenated oils can remain solid or semisolid at room temperature and have a comparatively long shelf life, because they are more stable against autoxidation than the original oils.

The hydrogenation of unsaturated organic compounds, that is treatment with gaseous hydrogen in the liquid phase on solid metal catalysts (based on Cu, Fe, Co and Ni) to decrease the number of double bonds, was developed at a time when there was demand for solid fats. The procedure developed by Wilhelm Norman in 1901 introduced a nickel-based catalyst on a solid carrier. This active catalyst was formed by depositing partially reduced nickel from nickel oxide (NiO) on a diatomaceous earth. Molecules of the reaction partners, hydrogen and triacylglycerols, are adsorbed, through the double bond, onto the surface of the active metal. The hydrogenation is then conducted via the adsorbed free radicals of the fatty acids bound in the triacylglycerols that form a complex with the catalyst. This complex reacts with hydrogen and dissociates to give a hydrogenated fatty acid and the catalyst (Figure 3.23). In addition to the reduction of the double bond, positional isomerisation (migration of the double bond) and, in particular, geometric isomerisation (*cis*–*trans* isomerisation) also proceed. Unsaturated fatty acids in the *trans* configuration have melting points much higher than the corresponding *cis* unsaturated fatty acids, thus contributing to the consistency of solid hydrogenated oils. The extent of *cis*–*trans* isomerisation and positional isomerisation is often even greater than that of the hydrogenation reactions, for example in the production of fats for making candies and icings. It is therefore more representative to describe this situation by the term **hardening**, which includes both hydrogenation and isomerisation reactions.

The hydrogenation reaction is controlled by the nature of the fatty acid to be hydrogenated, the nature and concentration of the catalyst, hydrogen pressure, reaction temperature and degree of agitation. Hydrogenation of fatty acids in triacylglycerols is not a function of their location. The individual steps taking place in a hydrogenation process include transfer and/or diffusion of

[19]The thick layer of fat under the skin of marine mammals provides an energy store and an effective insulating layer, preventing the loss of body heat to the surrounding water. Blubber has been used (when boiled down) in food processing, engineering, cosmetics and printing, but all of these products can now be produced synthetically.

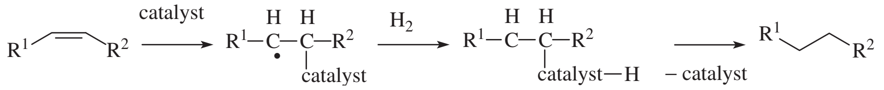


Figure 3.23 Scheme of hydrogenation of unsaturated fatty acids and their esters.

reactants and products to and from the bulk of the liquid oil phase and the outer surface of the catalyst, adsorption, hydrogenation and isomerisation, desorption and transfer. Adsorption of the reactants on the catalyst surface is important to control the selectivity of the hydrogenation and isomerisation reactions during the process. Once oil has been hydrogenated, the result is a substance that is made up of stiffened fat molecules. Because the crucial aspects are the physical properties of hydrogenated oil (melting point and consistency), the hydrogenation of double bonds is usually incomplete and only 25–40% of double bonds are reduced. This procedure is called partial catalytic hydrogenation.

A significant property of partial catalytic hydrogenation is the selectivity of hydrogenation. The hydrogenation of trienoic acids is the fastest reaction. The hydrogenation of dienoic fatty acids is a slower reaction, and the slowest reaction is the hydrogenation of monoenoic fatty acids (in oils originally containing linolenic, linoleic and oleic acids). The selectivity of hydrogenation can be expressed as the ratio of the reaction (hydrogenation) rate of linoleic acid transformed into monoenoic acids to the reaction rate of monoenoic acids transformed into saturated acids (Figure 3.24). In a very selective hydrogenation ($k_1 > k_2$), for example, linoleic acid in sunflower oil is selectively hydrogenated to octadecenoic acid isomers (incorrectly called isooleic acids). In a non-selective hydrogenation ($k_1 = k_2$ or $k_1 < k_2$), a larger amount of stearic acid is formed. Octadecenoic acids are transformed into stearic acid faster than they are generated. The selectivity of triacylglycerols defines the resulting distribution of stearic acid in the triacylglycerol molecules. It is maximum at a uniform distribution of stearic acid in triacylglycerols and zero if stearic acid accumulates in the form of tristearoylglycerols that have a high melting point, which causes the hydrogenated tallowy flavour of hydrogenated oils.

Oils previously used in blends of emulsified and edible fats were only partially hydrogenated (their melting point did not exceeded the temperature of the oral cavity, because they would then have had a tallowy flavour). The process ends with the reduction of the double bonds to about 30% on using selective conditions, where two double bonds of trienoic fatty acids and one double bond of dienes were hydrogenated, which ensured a minimum increase in the stearic acid content. The main reaction products of partially hydrogenated oils are monoenoic fatty acids, in which the ratio of *cis* and *trans* isomers is about 30 : 70. The content of saturated acids in partially hydrogenated oils does not increase significantly, while the *trans* unsaturated fatty acids content reaches several tens of percent. The predominating *trans* isomer is elaidic acid. Examples of the composition of hydrogenated oils are listed in Table 3.44.

The extent of geometric isomerisation during hydrogenation can be somewhat reduced by using catalysts based on precious metals. The resulting products are usually liquid and contain lower amounts of *trans*-monoenoic fatty acid and of stearic acid. The *trans* isomers content still remains at 1–10%.

Unfortunately, a significant degree of *cis*–*trans* isomerisation during hydrogenation of oils simultaneously leads to the formation of *trans* fatty acids in some triacylglycerols, which represent a

Table 3.44 Composition of groups of fatty acids in partially hydrogenated fats (% of total fatty acids).

Fatty acid types	Hydrogenated sunflower oil	Hydrogenated soybean oil	Hydrogenated rapeseed oil
Saturated	18–28	20–30	12–20
cis-Monoenoic	15–20	10–20	10–23
trans-Monoenoic	35–45	22–45	20–40
Dienoic	1–15	1–10	1–8
Trienoic	0	traces–0.1	traces–0.4

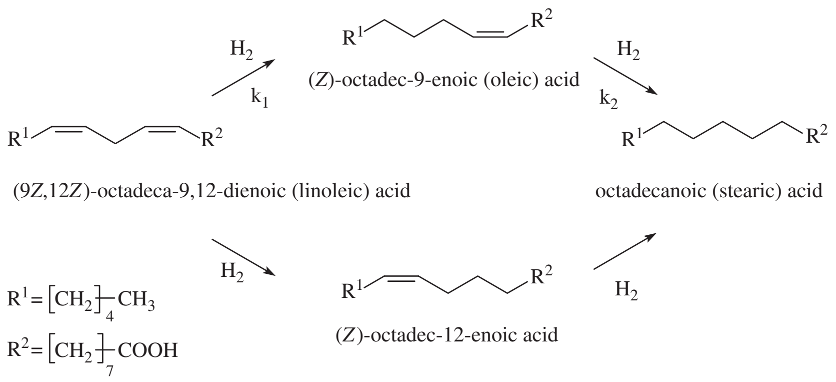


Figure 3.24 Course of selective hydrogenation of linoleic acid.

serious risk of cardiovascular diseases. This negative effect of *trans* isomers of fatty acids occurring in partially hydrogenated oils has stimulated efforts to achieve their full elimination, which for industry means producing no partially hydrogenated fats. Major manufacturers have actually abandoned this technology and have been forced to replace it by another process, which is usually a transesterification procedure in combination with fractionation of triacylglycerols (fractional crystallisation). Hydrogenation of oils for food purposes is now only used for the production of **fully hydrogenated fats**, where the residual content of monoenoic fatty acids is 1–1.5% and the residual content of *trans*-monoenoic fatty acids is 0.7–1.0%. Owing to the long reaction time, hydrolysis of triacylglycerols on the surface of the Ni-catalyst occurs (the water content in oil is 0.03–0.05%, which corresponds to one mole of water per 40 moles of triacylglycerols) and there is an increase in the free fatty acids, which must be removed by refining. These fats, due to their very high melting points, have no direct use and are employed as raw materials for transesterification.

3.8.1.7.2 Side reaction during hydrogenation

During partial catalytic hydrogenation, various side reactions also proceed, the most important of which is double bond geometric and positional isomerisation, includes the special case of conjugated double bond formation. After adsorption on the surface of the catalyst, the fatty acid is not necessarily hydrogenated because it is often desorbed just before the adsorbed double bond reacts with hydrogen. The double bond adsorbed on the surface of the catalyst can isomerise and the fatty acid can be desorbed as a *trans* fatty acid. Indeed, in partially hydrogenated fats, up to 70% of double bonds of fatty acids can be in the *trans* configuration. Double bonds can also migrate to different positions in the chain (Figure 3.25).

For example, partially hydrogenated oleic acid contains isomers with double bonds in positions Δ7 to Δ11. The unsaturated fatty acids content is 42% of the total unsaturated fatty acids, in which the main geometrical isomer is elaidic acid (13%), followed by Δ8 (10%), Δ10 (10%), Δ7 (7%) and Δ11 (7%) positional isomers. In natural menhaden oil mainly *cis*-Δ11 isomers occur, but in hydrogenated oil positional isomers are present with double bonds in positions Δ3–Δ17, of which *cis*-Δ6, *cis*-Δ9 and *cis*-Δ11, *trans*-Δ11 and *trans*-Δ13 isomers predominate. Partially hydrogenated vegetable oils and fats contain mainly isomers of octadecenoic acids. The representation of individual isomers may vary depending on the fatty acid composition of the starting oil. The hydrogenated oils, originally containing oleic, linoleic and linolenic acids, contain Δ4–Δ16 fatty acid isomers. Their distribution is monomodal and the major positional isomer is elaidic acid. Other isomers are *trans*-Δ10, *trans*-Δ11 and *trans*-Δ12 isomers. Distribution of *trans*-octadecenoic acids in hydrogenated fats is very different from their distribution in cows' milk fat (Table 3.45). The nutritional value of various positional isomers has not been fully investigsted.

3.8.1.7.3 Hydrogenation of carboxyl or ester groups

At high temperatures, under pressure and using Adkinson catalysts from intermetallic compounds, the fatty acid carboxyl groups are

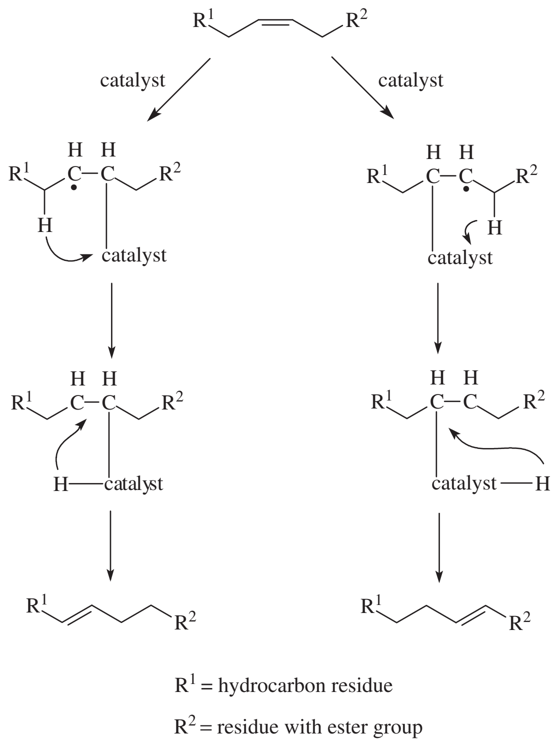


Figure 3.25 Positional isomerisation of a double bond during unrealised hydrogenation.

Table 3.45 Relative representation of *trans*-octadecenoic fatty acids in margarine blends containing partially hydrogenated fats in comparison with cows' milk fat (% of total *trans*-octadecenoic acids; indicated are ranges of values and median values).

Double bond position	Partially hydrogenated vegetable oil	Cows' milk fat
Δ4	0.0-1.6/0.3	0.9-1.7/1.2
Δ5	0.3-1.3/0.7	0.7-1.4/1.0
Δ6-8	9.4-24.1/18.5	3.8-5.0/4.5
Δ9	9.1-34.2/23.7	4.3-6.9/5.8
Δ10	8.7-26.1/20.7	2.7-5.3/4.3
Δ11	6.1-21.1/13.4	42.8-59.0/49.2
Δ12	5.1-18.8/10.9	4.2-6.1/5.4
Δ13-14	3.4-32.5/9.4	9.7-14.2/12.6
Δ15	0.5-5.2/1.5	6.9-8.7/7.5
Δ16	0.3-7.8/1.1	7.2-9.3/8.5
Total content (%)	0.2-25.9/9.3	3.2-5.9/4.3

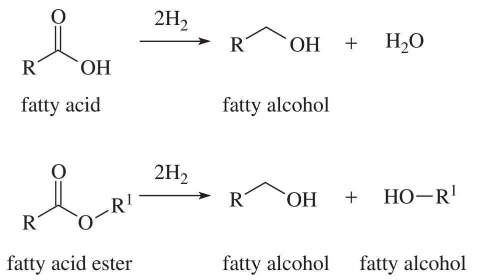


Figure 3.26 Hydrogenation of fatty acids and their esters.

also hydrogenated to primary hydroxyl groups and the hydrogenation of esters gives two alcohols (Figure 3.26). Alcohol R^2–OH is usually methanol. These hydrogenations are very important in oleochemistry for the production of fatty alcohols as hydrophobic substances for the production of surfactants (e.g. dodecanol and tetradecanol from coconut and palm kernel oils).

3.8.1.7.4 Enzymatic hydrogenation in ruminants

The diet of ruminants consists mainly of grass and leaves, where the lipids contain a large amount of linolenic acid. After ingestion, the food remains in the rumen for a long time before digestion. The rumen microorganisms breakdown the food to make it more palatable. The main concern is the cellulose, which is hydrolysed by cellulases. Rumen microorganisms also produce enzymes that hydrogenate polyenoic fatty acids. This enzyme-catalysed reaction proceeds by a mechanism similar to industrial catalytic hydrogenation. Analogous side reactions also occur that produce geometric and positional isomers of unsaturated fatty acids. Linolenic acid yields mainly (9*Z*,11*E*)-octadeca-9,11-dienoic (rumenic) acid, which exhibits antiatherogenic effects. To a lesser extent (10*E*,12*Z*)-10-octadeca-10,12-dienoic acid is produced, which provides anticarcinogenic and other beneficial effects and acts as a precursor of (*E*)-octadec-11-enoic (*trans*-vaccenic) acid, the major positional isomer of *trans*-octadecenoic acids in cows' milk fat. Therefore, depot and milk fats of ruminants contain higher amounts of *trans*-unsaturated fatty acids (Table 3.17) than fats of monogastric animals or vegetable oils, which contain up to 2% of *trans*-fatty acids.

3.8.1.8 Oxidation reactions

3.8.1.8.1 Classification

Oxidation reactions of the fatty acid hydrocarbon chains are common to free fatty acids and their esters, such as triacylglycerols. Carboxyl groups of free fatty acids, however, accelerate the decomposition of fatty acid hydroperoxides and can react with some oxidation products. In foods, the following types of oxidation reactions of lipids may occur:

- **autoxidation by triplet oxygen**
- **oxidation by hydrogen peroxide or hydroperoxides**
- **oxidation by singlet oxygen** (mostly photooxidation)
- **oxidation catalysed by enzymes** (lipoxygenases)
- **oxidation by metals** in higher valency
- **oxidation by quinones** and related compounds.

3.8.1.8.2 Autoxidation

Autoxidation of fatty acids is the most common type of oxidation under conditions suitable for the processing or storage of food. At normal temperatures, only unsaturated fatty acids are oxidised by atmospheric (triplet) oxygen. At higher temperatures, such as during baking, frying and roasting, autoxidation of saturated fatty acids also proceeds.

Autoxidation of the hydrocarbon chain of fatty acids, and of many other hydrocarbons, is a radical chain reaction that proceeds in three stages. The simplified reaction mechanism is shown in Figure 3.27. The first stage of the reaction is the formation of a free **hydrogen radical** ($H^•$) and a free fatty acid radical ($R^•$) formed by homolytic cleavage of the covalent bond **H**–C of the hydrocarbon chain. Energy needed to break down the **H**–C bond of fatty acids may be obtained from various sources. It may be heat energy (from a heating source), ultraviolet or radioactive radiation, or visible radiation (in the presence of photosensitisers in the case of a two electron oxidation by singlet oxygen; see Section 3.8.1.8.4). The dissociation energy of the **H**–CH– bond in the middle of the saturated fatty acid chain is 422 kJ/mol, the dissociation energy of the H_3C–CH–**H** bond at the end of its chain is 410 kJ/mol, the dissociation energy of the methylene group adjacent to the double bonds, –**H**–CH–CH=CH–, is 322 kJ/mol, but the dissociation energy of the methylene group between two double bonds is only 272 kJ/mol. The fission of the fatty acid (lipid) molecule also occurs by reaction with another free radical (immediate reaction of the hydrocarbon chain directly with oxygen is thermodynamically difficult) or by reaction with transition metals (see Section 3.8.1.8.6). This first stage is called the **initiation stage** (initiation) of an autoxidation reaction.

The resulting fatty acid (lipid) free radical ($R^•$) is very reactive, so it easily combines with an oxygen molecule, which is actually

Initiation reaction

R–H (fatty acid residue in lipid) → $R^•$ (free radical)

Propagation reaction

formation of peroxyl radical

$R^• + O_2 \rightarrow R–O–O^•$

formation of hydroperoxide

$R–O–O^• + R–H \rightarrow R–O–OH + R^•$

Termination reaction

$2R^• \rightarrow R–R$

$R^• + R–O–O^• \rightarrow R–O–O–R$

$2R–O–O^• \rightarrow R–O–O–R + O_2$

Figure 3.27 Autoxidation chain reaction of lipids.

a biradical. This results in the formation of a peroxyl radical (R–O–O•). The peroxyl radical is also very reactive and splits off a hydrogen atom from another unsaturated fatty acid molecule with the formation of a **hydroperoxide** (R–O–OH) and another fatty acid free radical (R•). This second stage of the autoxidation reaction is called the **propagation stage** (propagation). The sequence of these two reactions in the propagation stage may be repeated just once or many more times; chains having more than 1000 steps have been observed. Therefore, autoxidation is called a radical chain reaction. The reaction of a fatty acid free radical with oxygen is much faster than the reaction of a peroxyl radical with a hydrocarbon chain of fatty acid. As a peroxyl radical reacts with a fatty acid molecule relatively slowly, this reaction therefore determines the reaction rate of the autoxidation.

If the concentration of free radicals in the reaction system is quite high, it is likely that two free radicals react together to form a relatively stable product, and the chain reaction ends. This third stage is called the **termination stage** (termination) of an autoxidation reaction.[20] With a limited supply of oxygen, when the rate of autoxidation depends on its partial pressure, the main radicals in the system are fatty acid radicals (R•) and the main termination reaction is their recombination. With an adequate oxygen supply, the reaction rate is independent of its partial pressure. This creates higher amounts of peroxyl radicals (ROO•) and the main termination reactions are then recombination of radicals of the fatty acids (R•) with peroxyl radicals and mutual recombination of peroxyl radicals.

Unsaturated fatty acids

Unsaturated monoenoic fatty acids split off hydrogen atoms relatively easily, at least the hydrogen from the methylene group that is adjacent to the double bond. Even less energy is needed to split hydrogen from dienoic and trienoic fatty acids. The primary reaction products of autoxidation are hydroperoxides. The number of double bonds remains unchanged, but the double bond is usually shifted by one carbon atom to either the carboxyl or methyl end; this is particularly easy in dienoic and trienoic fatty acids, because here the free radicals arise between two double bonds and are stabilised by mesomerism. The reaction with oxygen yields a peroxyl radical at one end of the mesomeric system, and the double bond is simultaneously rearranged from the *cis* configuration to the more stable *trans* configuration.

[20]Termination of hydrocarbon radicals is not the only recombination reaction. Other possibilities (depending on the structure of the radicals) are: (i) the termination by disproportionation, where hydrogen is eliminated, which yields an olefin ($R–CH_2–CH_2^{\bullet} \rightarrow R–CH{=}CH_2 + H^{\bullet}$), (ii) termination by transfer (e.g. in reaction with antioxidants), (iii) termination by recombination with a hydroxyl radical, which leads to an alcohol ($R-CH_2-CH_2^{\bullet} + HO^{\bullet} \rightarrow R–CH_2–CH_2–OH$) and (iv) further oxidation of the terminal hydroperoxide ($R-CH_2–CH_2^{\bullet} + O_2 \rightarrow R–CH_2–CH_2–O–O^{\bullet} \rightarrow R–CH_2–CH_2–O–OH$). In the terminal hydroperoxide, the link between oxygen atoms is then cleaved, which gives an alkanal ($R–CH_2–CH_2–O–OH \rightarrow R–CH_2–CH{=}O + H_2O$). Alternatively, the link between carbon atoms can be cleaved, which results in a shorter alkyl radical ($R–CH_2^{\bullet}$). Some of these reactions are discussed in relation to the secondary reactions of hydroperoxides.

Monoenoic acids

Autoxidation of oleic acid is illustrated in Figure 3.28 as an example of autoxidation of monoenoic acids. The C–**H** bond cleavage in the initial stage of the reaction happens in the vicinity of the double bond, which occurs on carbons C-8 or C-11 (the dissociation energy is 322 kJ/mol). The resulting fatty acid radical, which is called allylic radical, may or may not isomerise, therefore reactions of fatty acid radicals with oxygen in the propagation stage of the reaction produce four hydroperoxides (isomeric hydroperoxyoctadecenoic acids). At room temperature, the amounts are approximately the same, but are dominated to a certain extent by 8- and 11-hydroperoxides. These two hydroperoxides that are generated without the double bond shift, are a mixture of *cis*- and *trans*-isomers, while hydroperoxides formed by positional isomerisation of double bonds are *trans*-isomers.

Dienoic acids

The higher reactivity of the methylene group between two double bonds causes the oxidation process to take place almost exclusively at this carbon atom (dissociation energy is 272 kJ/mol). In the case of linoleic acid, the oxidation occurs at the C-11 methylene group and the main oxidation products are hydroperoxides with conjugated double bonds: (10*E*,12*Z*)- and (10*E*,12*E*)-9-hydroperoxyoctadeca-10,12-dienoic acids and also (9*Z*,11*E*)- and (9*E*,11*E*)-13-hydroperoxyoctadeca-9,11-dienoic acids. At room temperature or lower, it is mainly *cis,trans*- and *trans,cis*-hydroperoxides that are formed, while at higher temperatures mainly *trans,trans*-hydroperoxides arise.

11-Hydroperoxyoctadeca-9,12-dienoic acid (*cis*,*cis*-isomer) is formed at a low concentration (without double bond isomerisation). Radicals on carbons C-8 or C-14, adjacent to the double bond, are also formed to a lesser extent. As in the previous cases, these radicals give rise to the corresponding geometric isomers: 8-hydroperoxyoctadeca-9,12-dienoic and 10-hydroperoxyoctadeca-8,12-dienoic acids and 12-hydroperoxyoctadeca-9,13-dienoic and 14-hydroperoxyoctadeca-9,12-dienoic acids. These reactions of linoleic acid are given in Figure 3.29 and Figure 3.30.

Trienoic acids

In the initial stage of the autoxidation reaction, a linolenic acid C–H bond is cleaved preferentially at C-11 and C-14, that is, at the methylene groups located between two double bonds. The main oxidation products of autoxidation are four hydroperoxides with a system of two conjugated double bonds and an isolated third double bond, with hydroperoxyl groups located in positions C-9, C-12, C-13 and C-16. In fact, these hydroperoxides are a mixture of *cis*,*trans*- and *trans*,*trans*-isomers and the isolated double bond has the *cis* configuration (Figure 3.31).

The predominant oxidation products are 9-hydroperoxide and 16-hydroperoxide, as 12-hydroperoxide and 13-hydroperoxide are less stable and tend to transform into cyclic products (similarly to the reaction catalysed by lipoxygenase that produces prostaglandins). As in the case of linoleic acid, many other hydroperoxides are formed as minor products.

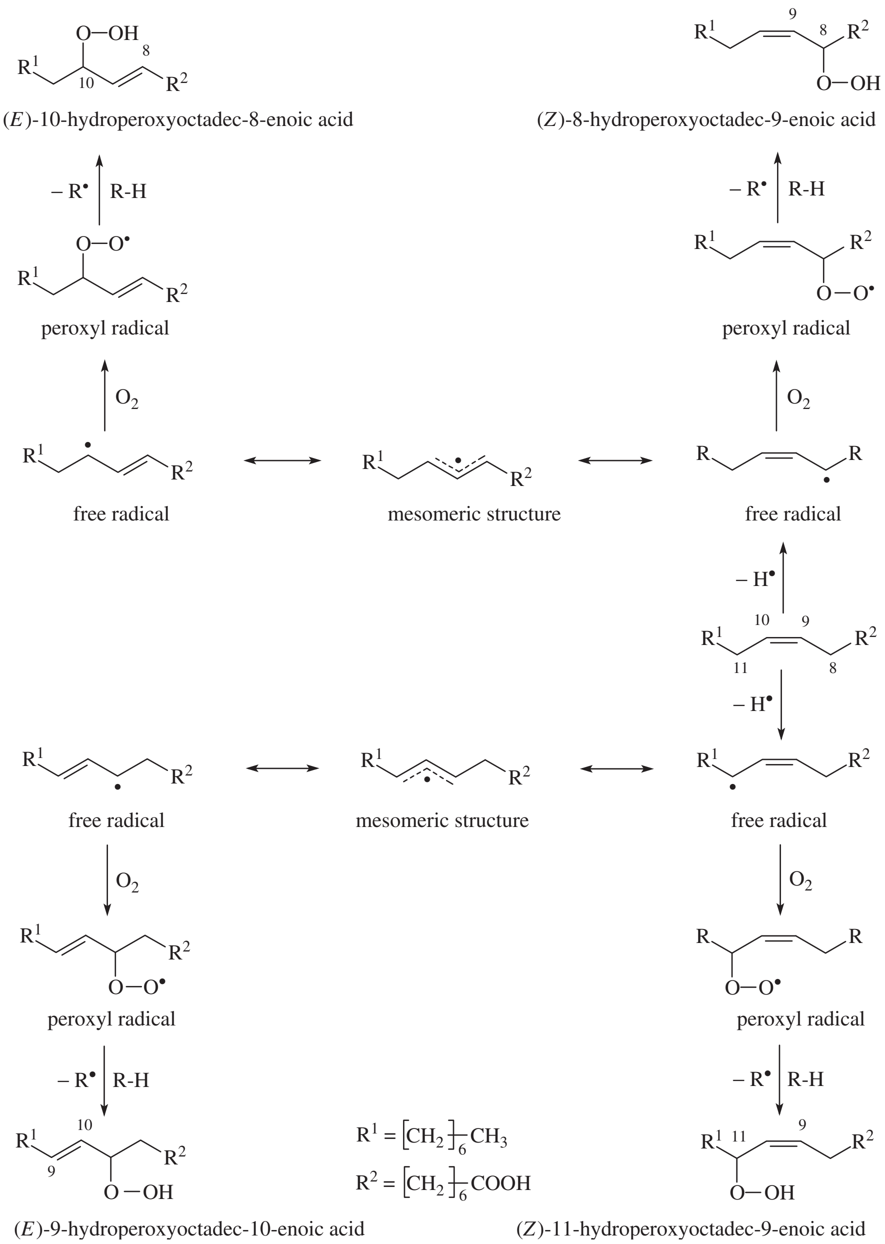


Figure 3.28 Autoxidation of oleic acid.

Saturated acids

Cleavage of the carbon–hydrogen bond in a saturated hydrocarbon chain requires a significant level of activation energy (422 kJ/mol), therefore this reaction does not come into practical consideration with normal temperatures. At higher temperatures however (corresponding to temperatures during baking, frying and roasting), this reaction is possible. In addition to the last carbon atom, all the other carbon atoms are almost equally prone to the creation of free radicals by the C–**H** bond cleavage. The third carbon and, to a lesser extent, the second and fourth carbons, are somewhat more reactive than the other carbon atoms of the hydrocarbon chain. The resulting free radicals bind oxygen through the formation of peroxyl radicals, and the reaction proceeds in a similar way to reactions of unsaturated acid peroxyl radicals, as already shown. Since saturated acids can form free radicals in many different positions along the hydrocarbon chain, the composition of the oxidation products is diverse.

Hydroperoxides

The primary products of autoxidation are sensorially indifferent hydroperoxides of fatty acids, that is products that have no taste and smell in comparison with other autoxidation products, such as aldehydes. Hydroperoxides, especially those of dienoic and trienoic acids, are very unstable compounds that decompose to either a hydrogen radical or a hydroxyl radical. In the former case, this decomposition yields a peroxyl radical, and in the latter

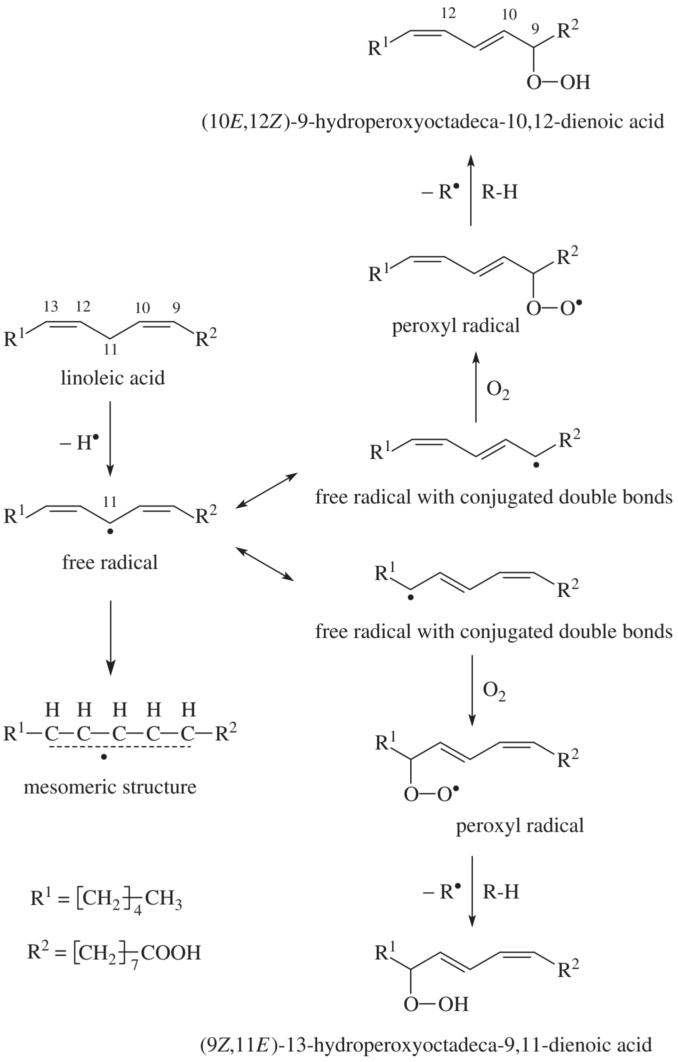


Figure 3.29 Main primary products of linoleic acid autoxidation.

case the reaction produces an **alkoxyl radical** (Figure 3.32). The decomposition of hydroperoxides, especially at higher concentrations, takes place via dimeric intermediates associated by hydrogen bonds (Figure 3.33). Both of these free radicals can initiate the chain radical reaction of fatty acids (Figure 3.32).

According to current knowledge, formation of the alkoxyl radical is favoured, as the O–O bond dissociation energy is about 184 kJ/mol, while the O–H bond dissociation energy is about 377 kJ/mol. During oxidation (and particularly in its advanced stages), these reactions are the major initiation reactions. The reaction of a fatty acid with a peroxyl radical gives fatty acid hydroperoxide, while reaction with an alkoxyl radical produces a hydroxy acid.

The timeline of an autoxidation reaction is shown in Figure 3.34. At the beginning, the reaction is usually initiated by heat or radiation, so the initial reaction rate is low. This reaction stage is called the **induction period**. Hydroperoxides gradually accumulate in the system, which causes formation of other radicals, thus the initiating reaction rate increases with an increasing concentration of hydroperoxides. Therefore, this reaction is called an autocatalytic reaction. If there is sufficient oxygen, the reaction rate increases rapidly until, at some point, the rate of decomposition of hydroperoxides exceeds the rate of their formation, and then the amount of hydroperoxides gradually decreases. During deceleration of the formation of additional hydroperoxides, their decomposition by the above mechanism becomes more important. Decomposition of hydroperoxides does not depend on the presence of oxygen or of unreacted lipids. At higher temperatures, such as during deep fat frying, hydroperoxides do not accumulate in fat, because they decompose at about 150 °C.

Secondary autoxidation products

Hydroperoxides of fatty acids and their radicals can in principle react in three ways in secondary reactions:

- reactions that do not change the number of carbon atoms in the molecule (e.g. the formation of cyclic peroxides and endoperoxides, epoxy acids, hydroxy acids and oxoacids);
- reactions involving decomposition of molecules, producing products with fewer carbon atoms (formation of aldehydes, hydrocarbons or oxoacids);
- polymerisation reaction in which the number of carbons in the molecule increases.

Cyclic peroxides and endoperoxides

Hydroperoxides of polyenoic fatty acids with three or more double bonds in the molecule, which have a system of conjugated double bonds in position β to hydroperoxide group, are very unstable compounds. They tend to form more stable cyclic six-membered peroxides derived from 1,2-dioxane by 1,4-cyclisation, five-membered peroxides derived from 1,2-dioxolane by 1,3-cyclisation and endoperoxides.

As an example, the formation of 1,2-dioxanes from 12-hydroperoxyoctadeca-9,13,15-trienoic acid derived from linolenic acid is shown in Figure 3.35. 1,2-Dioxanes are unstable compounds and decompose to low molecular weight flavour-active products. For example, 10-hydroperoxy-9,12-peroxyoctadeca-13,15-dienoic acid is decomposed into octa-3,5-dien-2-one. The mechanism of formation of 1,2-dioxolanes, cyclic peroxides and cycloendoperoxides from 13-hydroperoxyoctadeca-9,11,15-trienoic acid is shown in Figure 3.36. Peroxohydroperoxides of the 1,2-dioxolane type are considered to be the main precursors of toxic malondialdehyde (see Section 3.8.1.12.1).

Epoxy acids, hydroxy acids and oxoacids

Hydroperoxides, or strictly peroxyl radicals, readily react with unsaturated double bonds of olefins to form epoxide derivatives. The addition of a peroxyl radical to the double bond of an olefin can proceed as an intermolecular reaction (Figure 3.37).

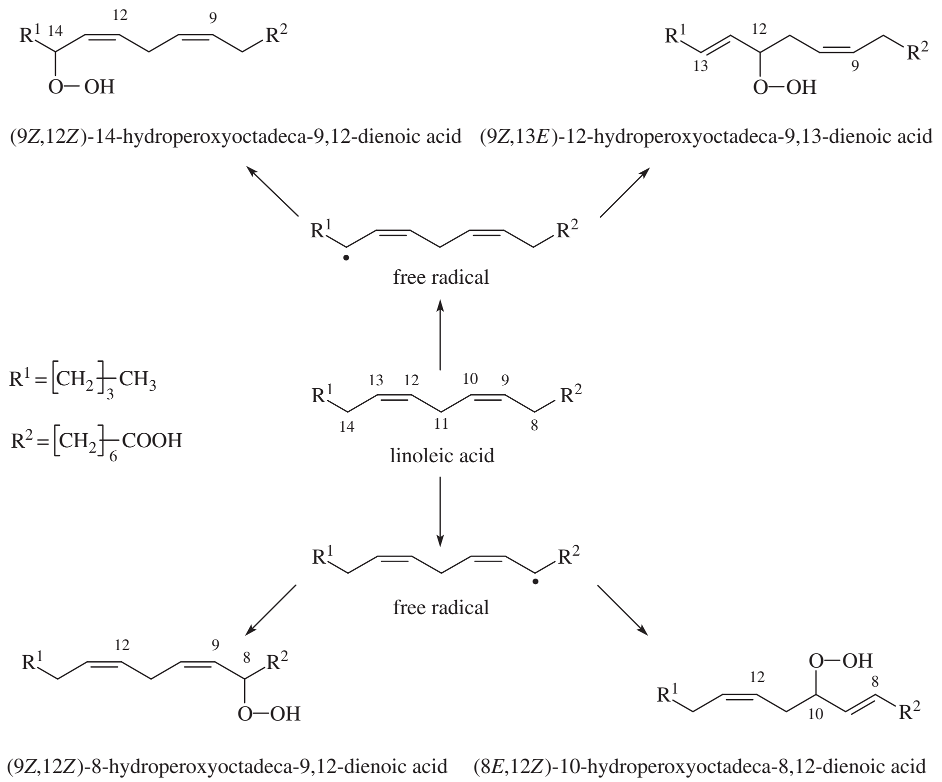


Figure 3.30 Minor primary products of linoleic acid autoxidation.

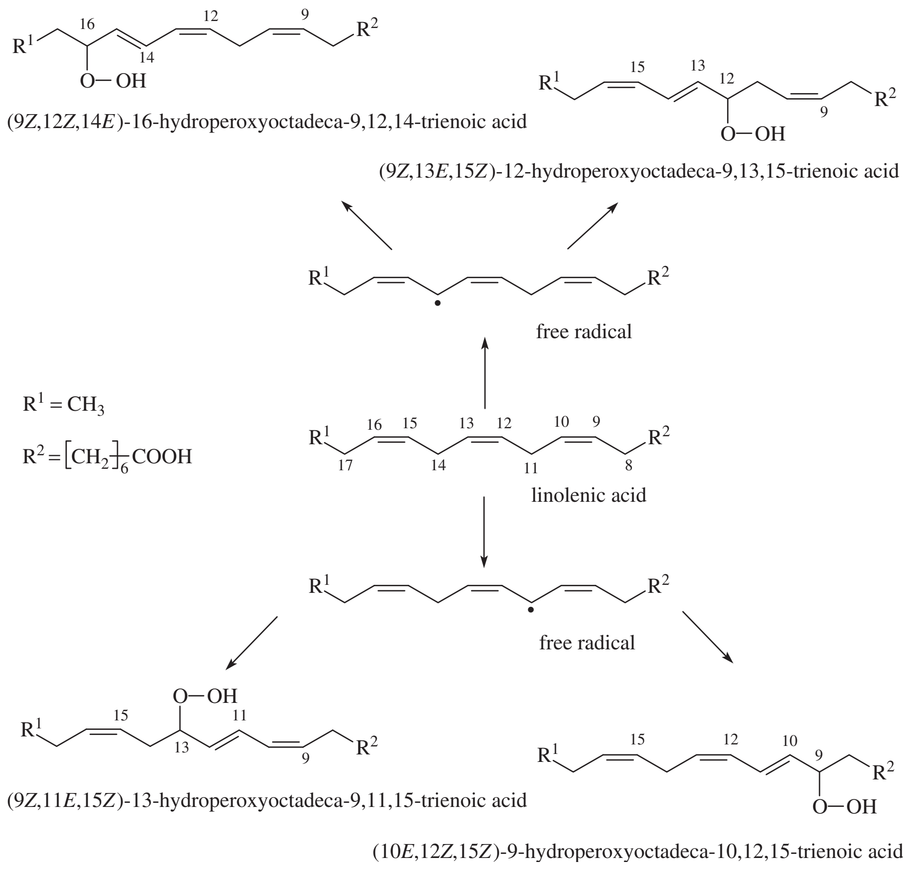


Figure 3.31 Main primary products of linolenic acid autoxidation.

$$R{-}O{-}OH \rightarrow R{-}O{-}O^{\bullet} + H^{\bullet} \qquad R{-}H + R{-}O{-}O^{\bullet} \rightarrow R^{\bullet} + R{-}O{-}OH$$

$$R{-}O{-}OH \rightarrow R{-}O^{\bullet} + HO^{\bullet} \qquad R{-}H + R{-}O^{\bullet} \rightarrow R^{\bullet} + R{-}O{-}OH$$

$$2\,R{-}O{-}OH \rightarrow R{-}O{-}O^{\bullet} + R{-}O^{\bullet} + H_2O$$

Figure 3.32 Hydroperoxides as initiators of autoxidation reactions.

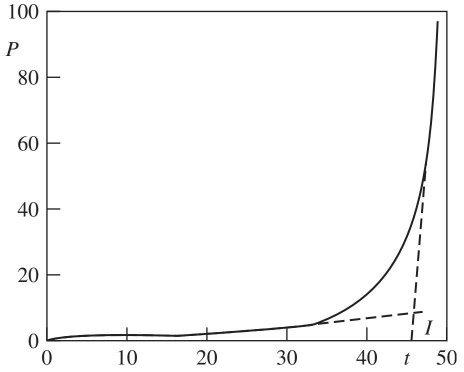
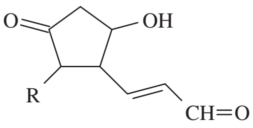


Figure 3.34 Course of autoxidation reaction of sunflower oil at 40 °C: P = amount of fatty acid hydroperoxides in milliequivalents of oxygen per kg (peroxide number), t = duration of the autoxidation in days, I = induction period.

Similarly, epoxides are also formed during the reaction of olefins with hydrogen peroxide. Secondary reactions of oxidised polyenoic acids produce red-coloured cyclopentane derivatives (**3-137**) causing adverse colour changes to stored fish.



3-137, cyclopentanolone derivative formed from oxidised fish lipids

A particularly important reaction of unsaturated fatty acids is an intramolecular reaction (Figure 3.38), occurring when epoxide arises from an alkoxyl radical. The resulting radical of epoxy acid reacts with oxygen to form a hydroperoxyl radical, which is decomposed via hydroperoxide to an alkoxyl radical. This alkoxyl radical provides, by recombination with a hydrogen radical, the corresponding hydroxy acid and elimination of a hydrogen atom yields the corresponding oxoacid. Somewhat more complicated is the intramolecular reaction of hydroperoxides with conjugated double bonds. Conjugated dienes, such as 13-hydroperoxyoctadeca-9,11-dienoic and 9-hydroperoxyoctadeca-10,12-dienoic acids, arise as major autoxidation products of linoleic acid. This reaction is illustrated in Figure 3.39 in the example of 13-hydroperoxyoctadeca-9,11-dienoic acid. Decomposition of this hydroperoxide via alkoxyl radicals creates 13-hydroxyoctadeca-9,11-dienoic- and 13-oxooctadeca-9,11-dienoic acids. Oxidation and other reactions of the alkoxyl radical give rise to two isomeric hydroperoxy acids: 12,13-epoxy-11-hydroperoxyoctadec-9-enoic and 12,13-epoxy-9-hydroperoxyoctadec-10-enoic acids. Decomposition of these epoxyhydroperoxy acids produces, analogously, epoxyhydroxy acids and epoxyoxoacids.

The alkoxyl radicals resulting from the decomposition of hydroperoxides with conjugated double bonds form by 1,4-addition radicals of dihydrofuran derivatives. These radicals are stabilised, in the same way as 1,2-epoxide radicals, by reaction with oxygen and subsequent reactions provide dihydrofuran derivatives. Radicals of dihydrofuran derivatives can also react with oxygen to form a peroxyl radical, which cleaves another fatty acid molecule and is transformed into the corresponding hydroperoxide from which, via an alkoxyl radical, hydroxy acids and oxoacids are formed (Figure 3.40).

Cyclic peroxides (1,2-dioxolane derivatives) provide substituted derivatives of tetrahydrofuran by ring cleavage and rearrangement (Figure 3.41). Dihydrofuran derivatives (so-called 1,4-epoxy compounds) can also be formed from unsaturated 1,2-epoxides by ring opening under acidic conditions and by cyclisation of the free radicals generated (Figure 3.42). This reaction is analogous to the formation of so-called 5,8-epoxides from 5,6-epoxides, which occurs in carotenoid pigments (see Section 9.9.1.2).

Epoxides are highly reactive compounds, especially at higher temperatures. They form dihydroxy derivatives with water, ethers with alcohols, esters with the carboxyl group of fatty acids, chlorohydrins (α-chlorohydroxy compounds) with hydrochloric acid and amino compounds with amines (Figure 3.43). Chlorinated hydroxy acids arise, for example, in polyvinyl chloride (PVC) by reaction of epoxidised soybean oil (used as a stabilizer of PVC) with hydrogen chloride (see Section 11.4.2.2.2).

Common oxidation products are derivatives with a hydroxyl group in the α-position to the oxo group, known as ketols. Ketols are formed in reactions of hydroperoxides with the neighbouring double bonds (Figure 3.44). They have significant reducing properties; their reactivity is close to the reactivity of aldehydes. The hydrolysis product of dihydrofuran derivative

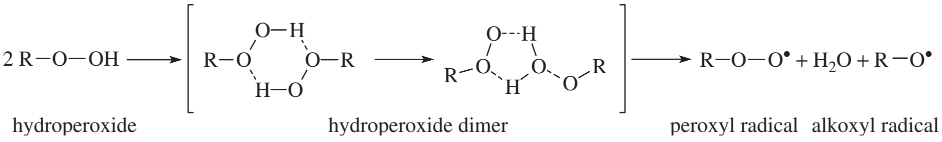


Figure 3.33 Decomposition of hydroperoxides to free radicals.

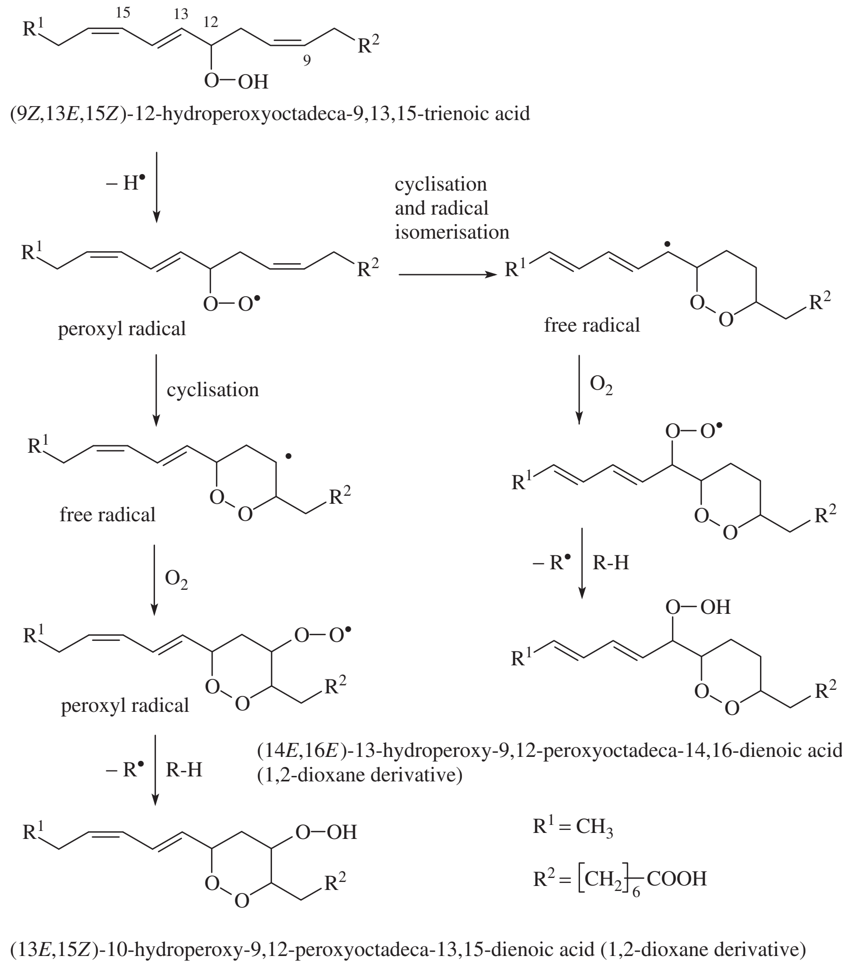
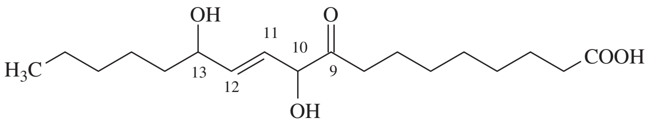


Figure 3.35 Formation of 1,2-dioxanes from 12-hydroperoxyoctadeca-9,13,15-trienoic acid.

of 9-oxo-10,13-epoxyoctadec-11-enoic acid, which is 10,13-dihydroxy-9-oxooctadec-11-enoic acid, is also a ketol. The structure of this ketol is shown in formula **3-138**.



3-138, (*E*)-10,13-dihydroxy-9-oxooctadec-11-enoic acid

Volatile secondary products

Another important group of reactions are those in which the hydrocarbon chain of the alkoxyl radicals cleaves to form low molecular weight products, mainly volatile and sensory active compounds. The cleavage takes place on both sides of the alkoxyl radicals (Figure 3.45). The composition of reaction products formed from alkoxyl radicals derived from unsaturated fatty acids depends on which carbon, next to the hydroperoxide group, the double bond is located. In addition to non-volatile oxoacids and hydroxy acids, the main volatile sensorially active decomposition products include saturated and unsaturated aldehydes and saturated and unsaturated hydrocarbons. As an example, the cleavage of two major hydroperoxides of linoleic acid, 13-hydroperoxy-9-octadeca-9,11-dienoic (Figure 3.46) and 9-hydroperoxyoctadeca-10,12-dienoic acids (Figure 3.47), are shown. Other hydroperoxides of unsaturated and saturated acids decompose in an analogous manner.

Hydrocarbons give a typical rancid flavour to slightly oxidised oils, while aldehydes carry a typical rancid flavour in the advanced stages of oxidation, and thus there is a close relationship between the aldehyde content and the intensity of the rancid flavour. The nature of the rancid odour changes according to the type of aldehyde. Alkanals and alk-2-enals impart a typical rancid flavour to oils but the sensory effects of alk-2-enals are more intense than those of alkanals. The oxidation of oils with higher linoleic acid contents produces alka-2,4-dienals, which are another important group of volatile oxidation products. In small amounts, they impart a fried flavour to oils, while at slightly higher levels the flavour resembles peanuts, and at even higher levels the flavour becomes unpleasant. Conjugated alkatrienals, forming during the oxidation of linolenic acid in oils, impart an off-flavour to oils, resembling varnish or fish oil. Substances that are less sensorially active than alkanals

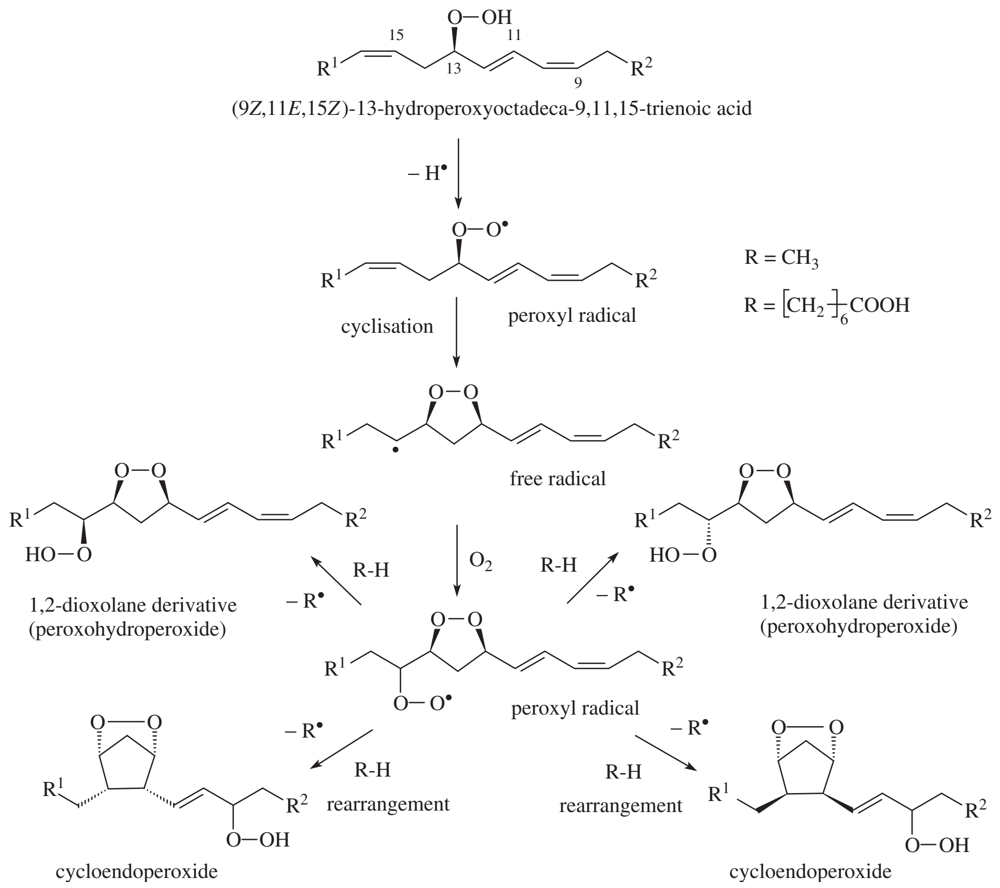


Figure 3.36 Formation of 1,2-dioxolanes from 13-hydroperoxyoctadeca-9,11,15-trienoic acid.

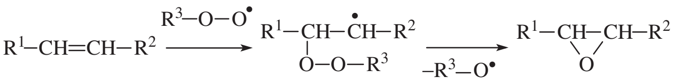


Figure 3.37 Formation of epoxide from peroxyl radical and olefins.

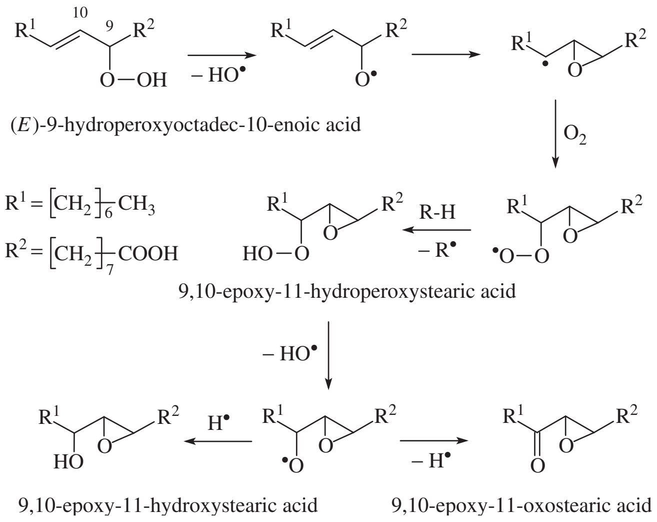
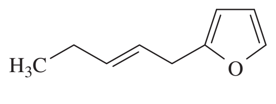


Figure 3.38 Formation of epoxy acids, hydroxyl acids and oxoacids from oleic acid 9-hydroperoxide.

and alkenals, but nevertheless are important reaction products, are alkan-2-ones, the odour of which is reminiscent of moldy cheese. An overview of the hydrocarbons and carbonyl compounds formed by decomposition of hydroperoxides of oleic, linoleic and linolenic acids is given in Sections 8.2.1.1.2 and 8.2.4.1.1.

A specific type of rancidity, known as reversion in the United States, occurs during the oxidation of soybean oil. Reversion is caused by some furan derivatives, such as 2-pentylfuran or (*Z*)- and (*E*)-2-(pent-2-en-1-yl)furan and other decomposition products of hydroperoxides. Pentylfuran arises (Figure 3.48) as a by-product of the decomposition of 9-hydroperoxyoctadeca-10,12-dienoic acid derived from linoleic acid via the terminal radical. The content of linoleic acid in soybean oil is about 51% of the total fatty acids. Similarly, both isomers (*cis* and *trans*) of 2-(pent-2-en-1-yl) furan (**3-139**) arise by decomposition of 9-hydroperoxyoctadeca-10,12,15-trienoic acid formed by autoxidation of linolenic acid.



3-139, (*E*)-2-(pent-2-en-1-yl)furan

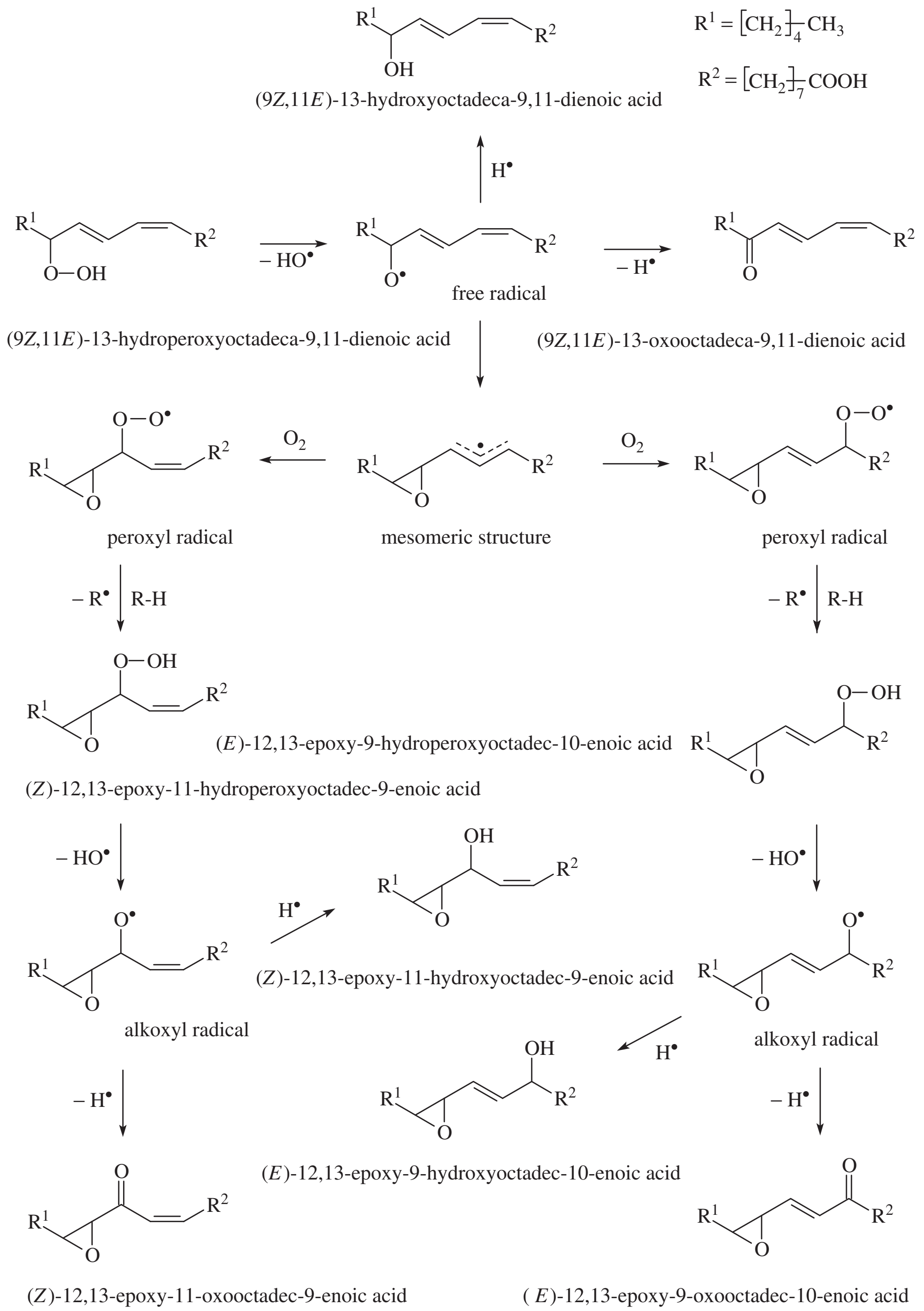


Figure 3.39 Formation of epoxy acids, hydroxy acids and oxoacids from linoleic acid 13-hydroperoxide.

Soybean oils used at present contan 7–10% linolenic acid. A number of other oxidation products of polyenoic fatty acids contribute to the smell of rancid (reverted) soybean oil.

Subsequent reactions of aldehydes

Aldehydes are very reactive compounds that react further in the oxidised fat. Their carbonyl group is oxidised to a carboxyl group and, as a rule, they are further oxidised in the hydrocarbon chain. Common reactions are aldolisation, retroaldolisation and reactions with other food components, such as proteins.

Alkanals

Autoxidation of the hydrocarbon chain and of the carbonyl group of alkanals creates, via unstable peroxy acids and 2-hydroperoxy derivatives, a mixture of products. The important final products are the corresponding fatty acids, formic acid and alkanals containing

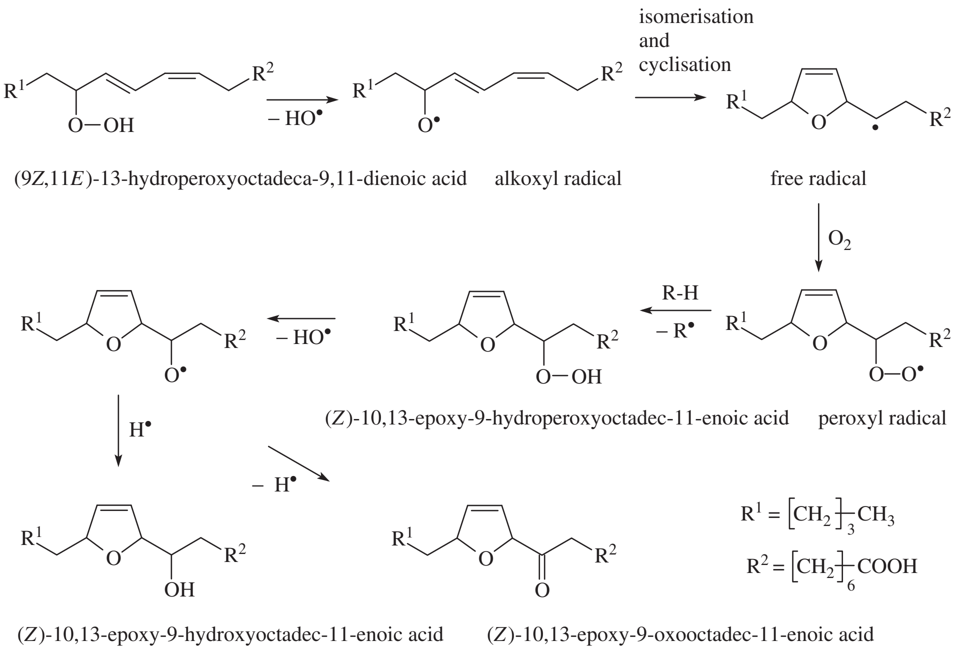


Figure 3.40 Formation of dihydrofuran derivatives from an alkoxyl radical of linoleic acid 13-hydroperoxide.

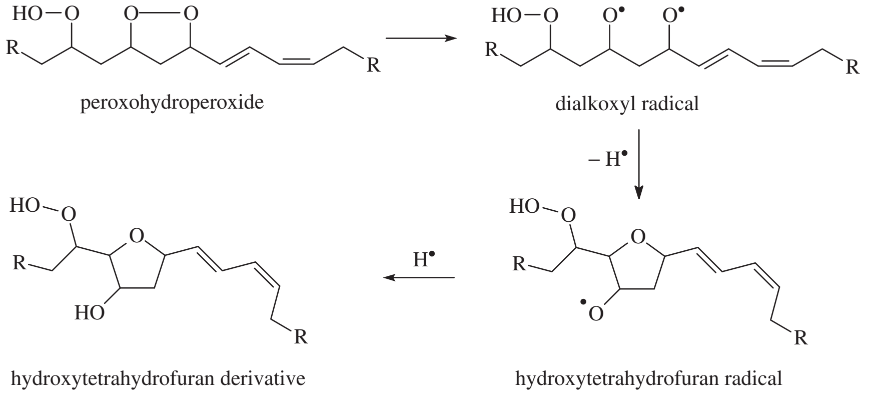


Figure 3.41 Formation of tetrahydrofuran derivatives from 1,2-dioxolanes.

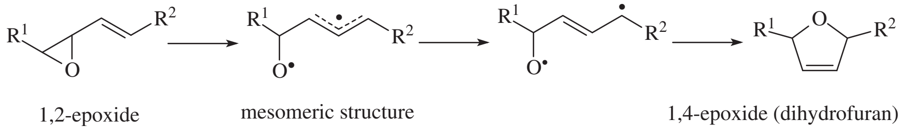


Figure 3.42 Formation of fatty acid 1,4-epoxides from 1,2-epoxides.

fewer carbon atoms per molecule than the parent compound (Figure 3.49). Lower alkanals, such as hexanal, also condense to trimeric 2,4,6-trialkyl-1,3,5-trioxanes (**3-140**).

Alk-2-enals

The continued autoxidation of alk-2-enals leads to a number of products that are formed by similar mechanisms that give rise to products arising from alkanals. An important product is highly reactive malondialdehyde (propanedial, also incorrectly known as malonaldehyde; Figure 3.50). Malondialdehyde exists predominantly in enol form, as a *cis*-isomer in organic solvents and as a *trans*-isomer in water (Figure 3.51).

As with alkanals, the carbonyl group of alk-2-enals is oxidised to a carboxyl group via the corresponding peroxy acid. Retroaldolisation creates acetaldehyde and an aldehyde containing two fewer carbon atoms, whereas aldolisation with acetaldehyde produces alka-2,4-dienals (Figure 3.52).

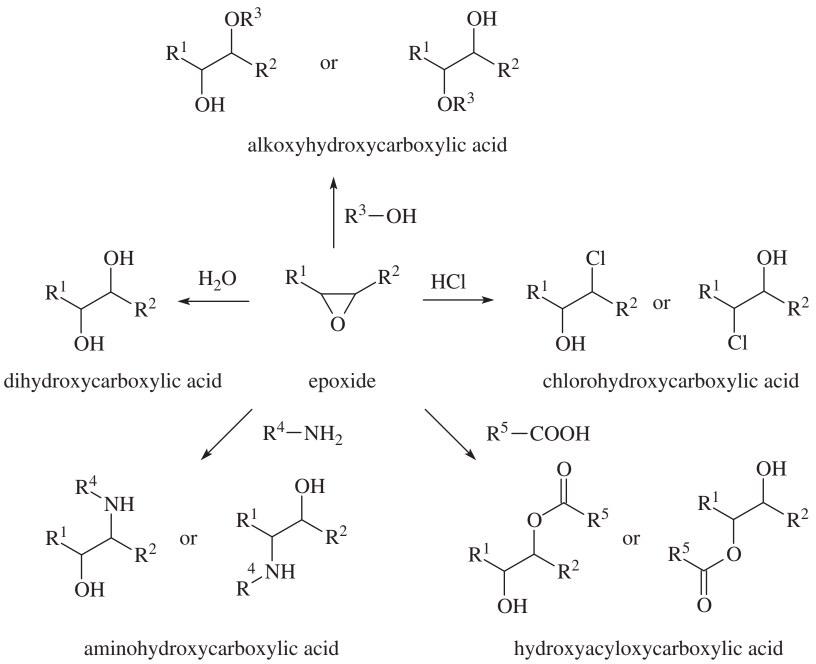


Figure 3.43 Important reactions of fatty acid epoxides.

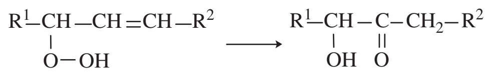
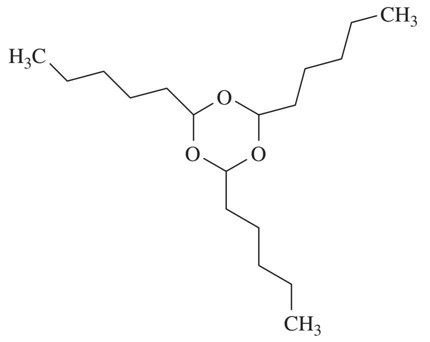


Figure 3.44 Formation of ketols by oxidation of fatty.



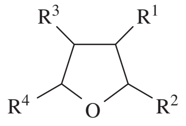
3-140, 2,4,6-tripentyl-1,3,5-trioxane

Alka-2,4-dienals

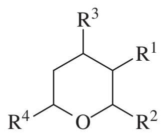
Autoxidation of alka-2,4-dienals accompanied by retroaldolisation leads temporarily to epoxides and finally to relatively stable end products. Similarly to alk-2-enals, the main reaction products include alkanals, alkenals, dialdehydes (including malondialdehyde) and hydrocarbons. Some secondary reactions of alka-2,4-dienals are shown in Figure 3.53.

Secondary polymeric products

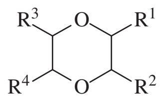
The third group of secondary reactions of oxidised fats are reactions in which the number of carbons in the molecule increases. In specialised areas these reactions are known under the term **oxypolymerisation** (oxidative polymerisation). Oxypolymerisation is one of the consequences of oxidative degradation of lipids. Free radical addition and sometimes cycloaddition reactions can be seen as the main causes of the production of oxidative polymers (oxypolymers) in triacylglycerols, especially if the polyunsaturated fatty acids content is high. Polymers are usually formed by reactions of two free radicals. If both are alkyl radicals, the formed dimers have the oxidised fatty acids bound by a single C–C bond between two carbon atoms. This reaction is described in more detail in Section 3.8.1.6. Alkyl radicals, of course, do not occur in high amounts and react slowly, so reactions between oxygen-containing radicals are more common (Figure 3.54). Most products contain ether bonds of the type C–O–C, although peroxide bonds of the type C–O–O–C can also occur. In theory, diperoxide bonds could also form, but they are unstable and their formation is accompanied by elimination of oxygen. Similarly, two fatty acids may also be linked to one another by two bonds, which yields tetrahydrofuran (**3-141**), tetrahydropyran (**3-142**) or dioxan derivatives (**3-143**). Usually,



3-141, dimer with tetrahydrofuran ring



3-142, dimer with tetrahydropyran ring



3-143, dimer with 1,4-dioxan ring

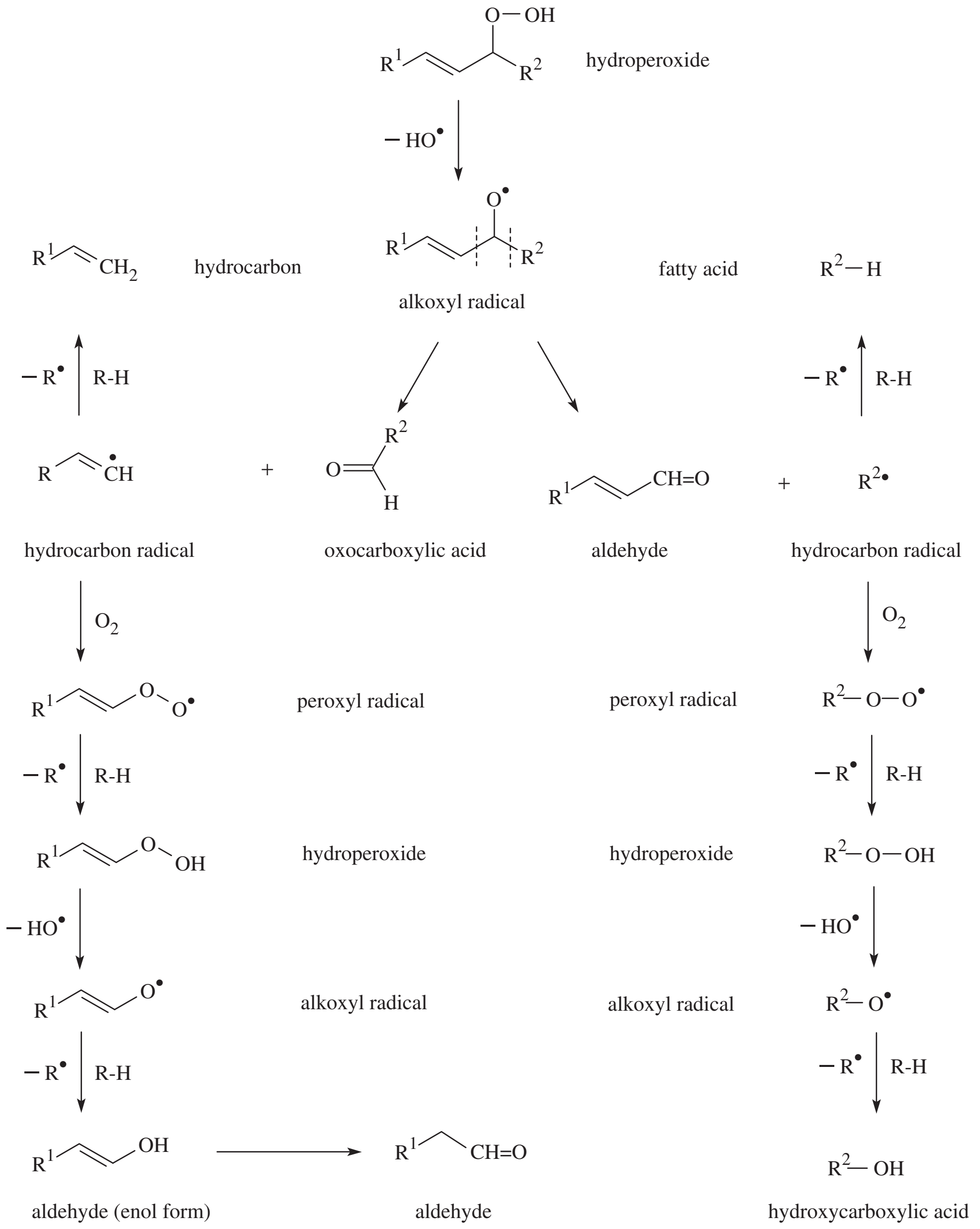


Figure 3.45 Fission of fatty acid hydrocarbon chain during hydroperoxide decomposition.

polymers are described by schematic formulae that indicate the type of link between the chains of original fatty acids (Figure 3.55).

Polymers may arise also by non-radical mechanisms. For example, polymers are formed by reactions of epoxides with hydroxyl groups or by reactions of epoxides with carboxyl groups of free fatty acids (Figure 3.43 and Figure 3.56). Many other types of polymerisation reactions have also been described.

Influence of reaction conditions

The rate of formation and decomposition of hydroperoxides is very dependent on the structure and concentration of reactants and on the reaction conditions, mainly on the temperature, but also on the concentration of oxygen, the surface area exposed to air, water activity, presence of prooxidants or antioxidants and so on.

Structure of acids

An important factor is the presence of reactive double bonds. Saturated fatty acids are practically stable during fat storage at normal temperatures. At temperatures around 20 °C, monoenoic fatty acids (such as oleic acid) are only oxidised very slowly, dienoic fatty acids (such as linoleic acid) are oxidised about ten times faster and trienoic fatty acids (such as linolenic acid) approximately 20 times faster. Arachidonic acid, as an example of tetraenoic fatty acids, is oxidised about 30–40 times faster. At temperatures above

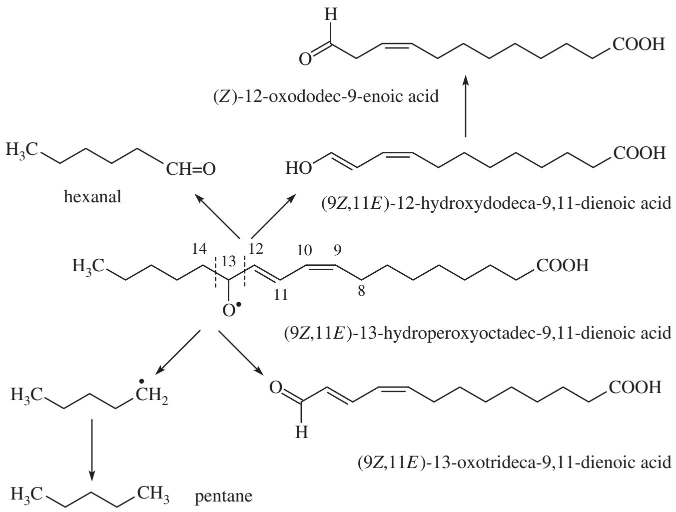


Figure 3.46 Fission of 13-hydroperoxyoctadeca-9,11-dienoic acid hydrocarbon chain.

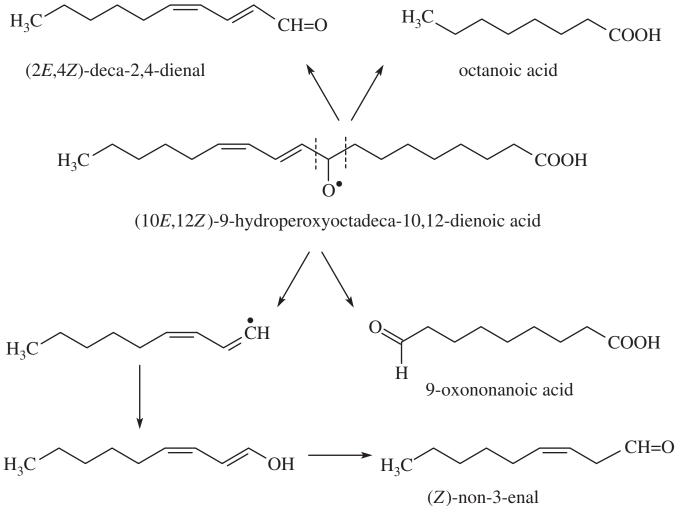


Figure 3.47 Fission of 9-hydroperoxyoctadeca-10,12-dienoic acid hydrocarbon chain.

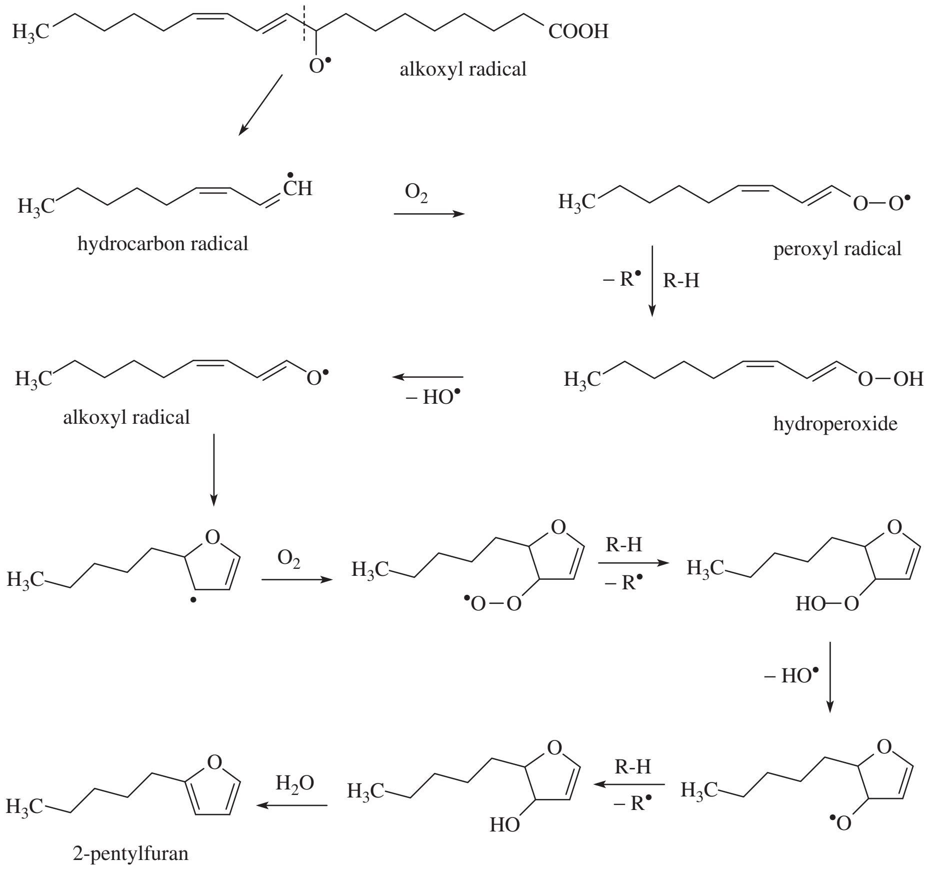


Figure 3.48 Mechanism of 2-pentylfuran formation.

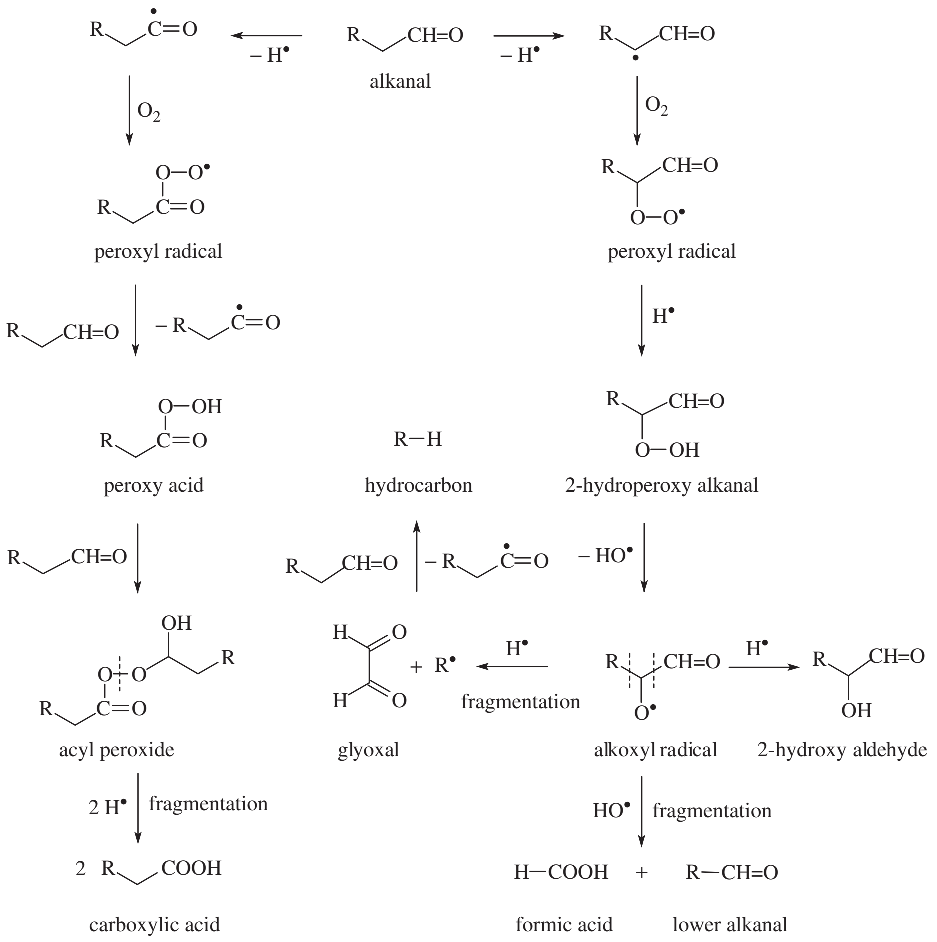


Figure 3.49 Oxidative fission of alkanals formed by decomposition of lipid hydroperoxides.

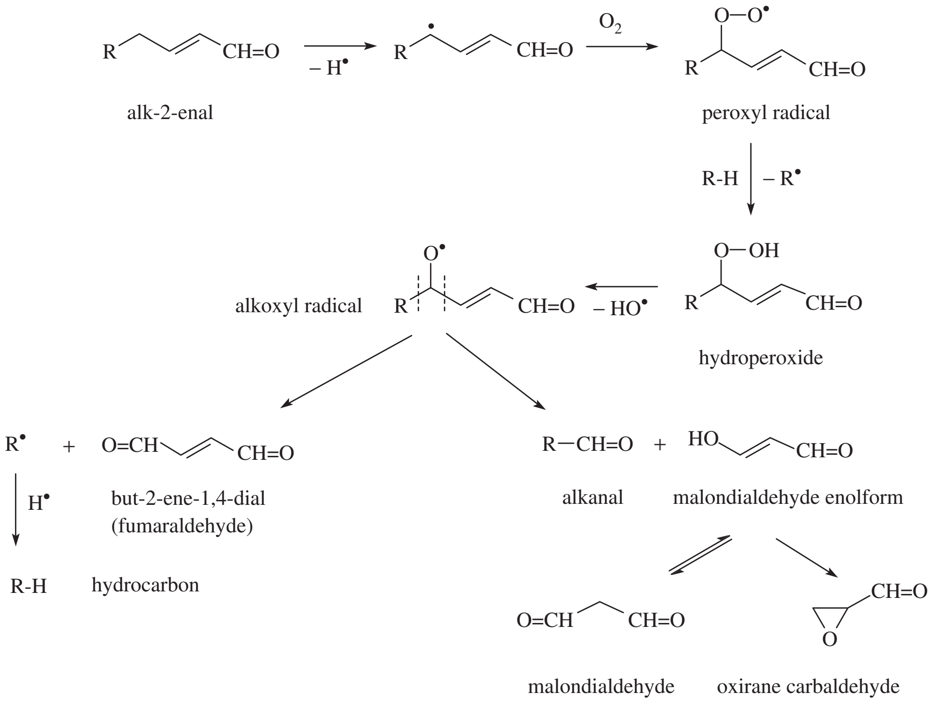


Figure 3.50 Oxidation fission of alk-2-enals formed by decomposition of lipid hydroperoxides.

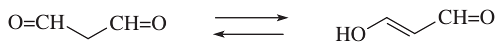


Figure 3.51 Isomerisation of malondialdehyde.

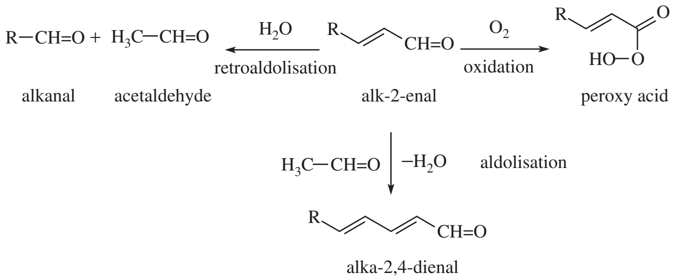


Figure 3.52 Oxidation and (retro)aldolisation of (*E*)-alk-2-enals.

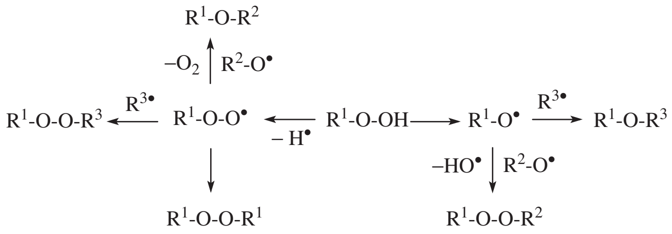


Figure 3.54 Secondary reactions of fatty acid hydroperoxides.

100 °C, all fatty acids are oxidised at about the same rates; *cis* isomers are generally less stable than *trans* isomers, conjugated dienes are less stable than dienes with isolated double bonds and free fatty acids are less stable than fatty acids bound in triacylglycerols.

A fatty acid's inclination to oxidation also depends on the presence of other fatty acids, which is important for natural fats and oils, where fatty acids occur as mixtures of esters. When, for example, higher amounts of linoleic acid hydroperoxides form, their decomposition leads to radicals, which are able to initiate oxidation of monoenoic fatty acids. Vegetable oils that contain less than 10% of polyenoic fatty acids are therefore very stable against oxidation under typical storage conditions. This is the main reason why oilseeds are today bred to have low linoleic acid and linolenic acid contents.

Temperature and oxygen concentration

A rise in temperature accelerates the oxidation process and thus also increases the content of hydroperoxides, but hydroperoxides are very unstable at high temperatures. Particularly unstable are the hydroperoxides of trienoic and other polyenoic fatty acids. With a

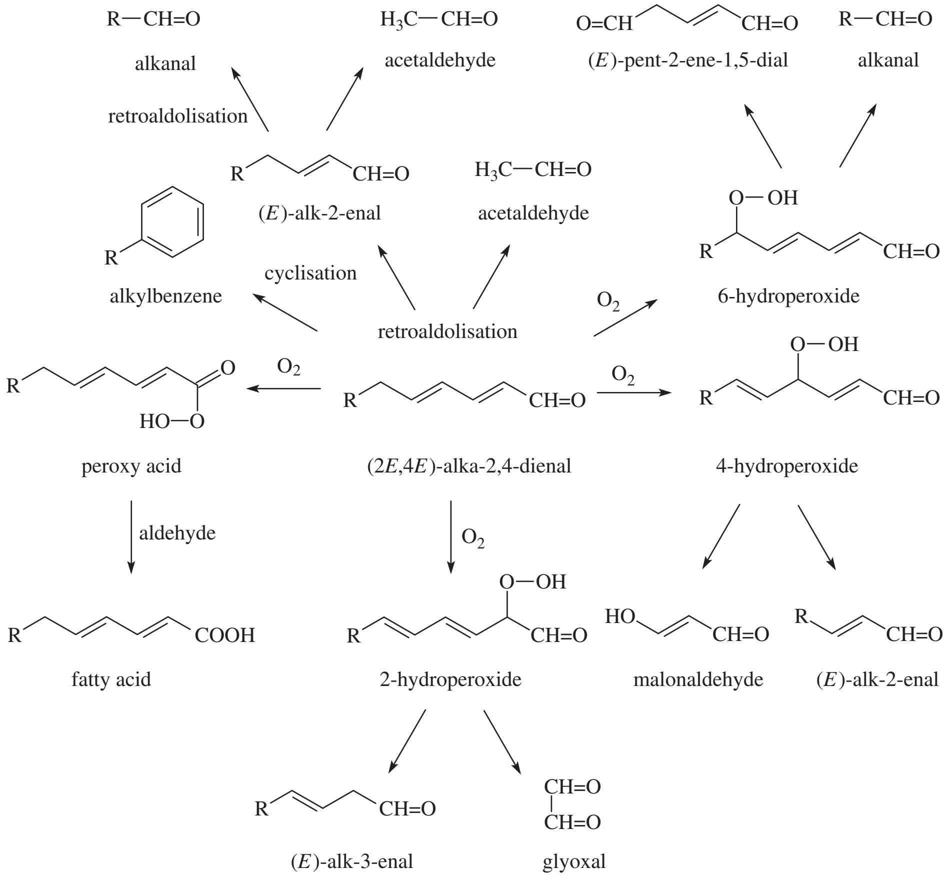


Figure 3.53 Secondary reactions of alka-2,4-dienals.

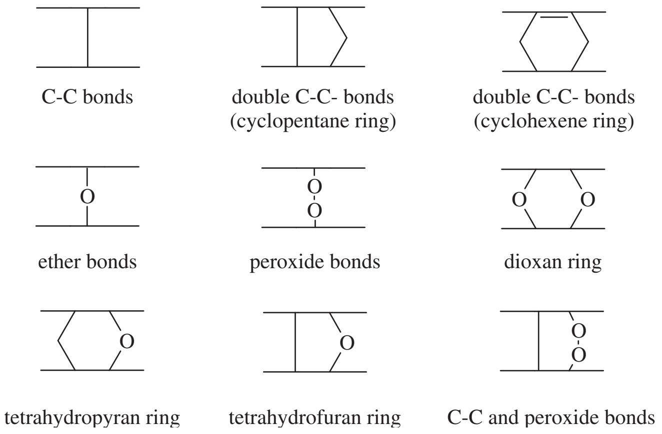


Figure 3.55 Schematic representation of bonds in dimers of oxidised fatty acids.

rise in temperature, the increase of the content of hydroperoxides is therefore soon compensated for by their faster decomposition. The maximum hydroperoxide content is achieved sooner, but it is lower and less distinct.

An important factor is the concentration of oxygen, as only oxygen molecules dissolved in the fat phase can react with free radicals. At higher temperatures, such as during frying, oxygen is consumed within a few minutes and the rate of oxidation is then determined only by the amount of oxygen that penetrates into the fat from the atmosphere by diffusion (diffusion of oxygen is also an important factor in autoxidation of fatty emulsions and dispersions). Under such conditions, the rate of formation of hydroperoxides and the rate of their decomposition are almost comparable, and their content remains approximately constant until the reactive unsaturated acids are oxidised. If a flow of air is introduced into fat, its oxidation is much faster, and the maximum content of hydroperoxides significantly increases.

Water activity

The rate of fat oxidation in foods depends greatly on water activity (see Section 7.9). In dry foods, oxygen can more easily penetrate into the material than in normal foods. The minimum rate of oxidation occurs in foods with water activity values around 0.3. This situation is explained by the decrease in catalytic activity of metals, by quenching free radicals and by formation of antioxidants in the Maillard reaction. In foods with higher water activity, the rate of oxidation is again higher, probably due to increased mobility of metal ions, which catalyse the autoxidation.

Pro-oxidants and antioxidants

Heavy metals with variable valencies have a significnt influence on the oxidation of unsaturated fatty acids and their derivatives, as they can accelerate the reaction, as pro-oxidants. On the other hand, antioxidants will retard the oxidation of fatty acids.

3.8.1.8.3 Oxidation by hydrogen peroxide

It has been shown that lipid hydroperoxides are easily decomposed to form free radicals that accelerate autoxidation reaction. Hydroperoxides can also oxidise unsaturated fatty acids by non-radical mechanisms, as mentioned in the secondary reaction of oxidised lipids. Also, hydrogen peroxide, which arises naturally in

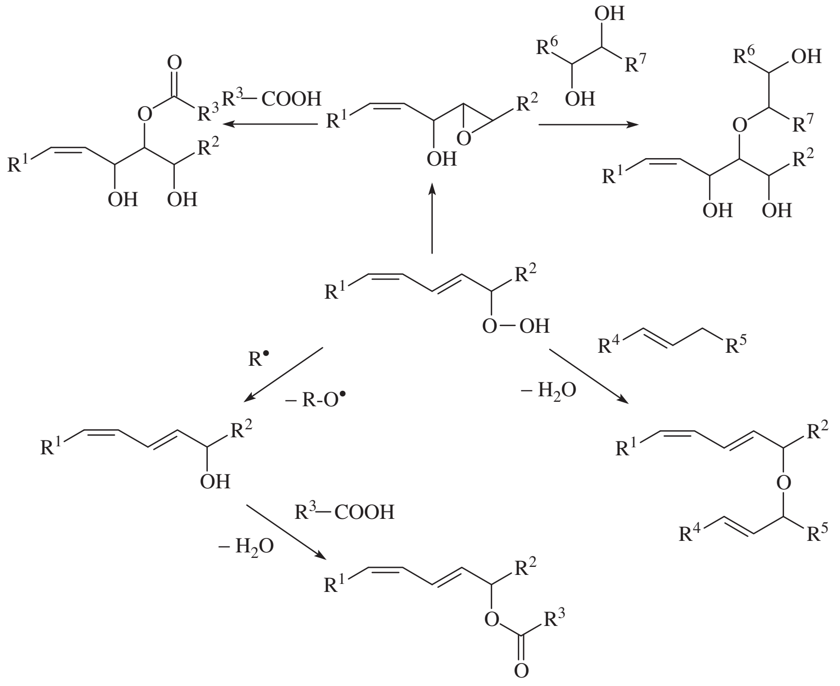


Figure 3.56 Examples of non-radical polymerisation during autoxidation of unsaturated fatty acids.

food processes, can oxidise unsaturated lipids. The primary oxidation product is epoxide, which is immediately hydrolysed to form a fatty acid dihydroxy derivative. For example, the oxidation of oleic acid by hydrogen peroxide yields 9,10-dihydroxystearic acid (Figure 3.57). These reactions are also of great importance in the development of vascular diseases.

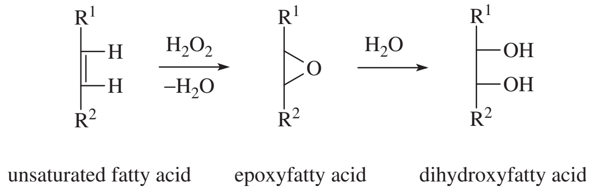


Figure 3.57 Oxidation of unsaturated fatty acids by hydrogen peroxide.

3.8.1.8.4 Oxidation by singlet oxygen

Excitation of ordinary triplet oxygen ($^3O_2$) yields reactive singlet oxygen ($^1O_2$), which can react with the unsaturated double bond of lipids and other unsaturated compounds.[21] Under the conditions eligible for the processing and storage of foods, singlet oxygen forms most often during irradiation in the presence of photosensitisers. With conventional unsaturated fatty acids, singlet oxygen reacts at least 1450 times faster than triplet oxygen. The relative reactivity of linolenate, linolate and oleate are roughly in the proportions 3.5:3:1.

Singlet oxygen adds to the double bond of fatty acids with the formation of unstable peroxide (formerly called moloxide) or a six-membered ring. The resulting intermediates rapidly decompose to form hydroperoxides (Figure 3.58). Analogously, isomeric hydroperoxide forms by reaction with a hydrogen atom of the methylene group at the carboxyl end of the fatty acid molecule. These hydroperoxides differ from hydroperoxides resulting from autoxidation reaction in the ratio of the individual positional isomers, since the primary mechanism of the formation of free radicals is different. For example, oxidation of oleic acid by singlet oxygen creates an equimolar mixture of 9- and 10-hydroperoxide, but isomeric hydroperoxides with the hydroperoxide group in positions C-8 and C-11 do not form. The products arising from linoleic acid by oxidation with singlet oxygen are a mixture that contains 66% of conjugated hydroperoxides (9- and 13-hydroperoxides) and 34% of unconjugated hydroperoxides (10- and 12-hydroperoxides). Linolenic acid oxidation by singlet oxygen produces a mixture containing 75% of conjugated hydroperoxides (9-, 12-, 13- and 16-hydroperoxides) and 25% of unconjugated hydroperoxides (10- and 15-hydroperoxides).

Photosensitisers

Photosensitisers are compounds that catalyse the oxidation of organic substances with oxygen when exposed to visible radiation. They act as carriers of absorbed energy that is transmitted to triplet oxygen and transformed to singlet oxygen.[22] The mechanism of their action is shown in Figure 3.59. The type I reactions, which occur, for example, in phenols, amines and quinones (easily oxidisable or reducible compounds), via direct interactions of a triplet photosensitiser with other molecules (R–H), which involves a transfer of a hydrogen atom or electron that produces free radicals ($R^\bullet$ and $^\bullet SH$). Triplet sensitisers are therefore photochemically activated free radical initiators. Reactions of type II describe the most common method of energy transfer from the excited triplet photosensitisers to triplet oxygen to form singlet oxygen. Less than 1% of triplet oxygen is simultaneously transferred to the superoxide anion (O_2^-) and oxidised form of the sensitiser (S^+).

Of the substances that are present in foods, the most important photosensitisers are chlorophylls, phaeophytins, haem pigments and riboflavin. In vegetable oils, chlorophylls (and their degradation

[21] According to the Pauli principle, each orbital of a molecule can be occupied by only two electrons (so-called paired electrons), which must have opposite spin directions also known as spins ($s_1 = 1/2$ and $s_2 = -1/2$). The spin of a molecule is the sum of the spins of unpaired electrons, which may be parallel or antiparallel. The ground state of most molecules is a singlet as their spin $S = s_1 + s_2 = 0$, the total magnetic moment $\boldsymbol{M} = 2S + 1 = 1$ and the molecule is diamagnetic. Excitation of such molecules occurring in the singlet state leads to a shift of one electron from the bonding orbital to free antibonding orbital with higher energy. Both electrons with opposite (antiparallel) spins, originally located in the same orbital, are now in different orbitals and have a parallel spin ($s_1 = s_2 = 1/2$). The resulting spin of the molecule $S = 1$ and the state of the molecule, which is paramagnetic, is a triplet, since the total magnetic moment $\boldsymbol{M} = 2S + 1 = 3$.

Oxygen is an exception to this general rule. Atmospheric oxygen, O_2, exists in the triplet state ($^3O_2$). It has 12 valence electrons, of which two electrons with higher energy and parallel spin are located in different orbitals. It is actually a biradical (•O–O•). Excitation of triplet oxygen creates singlet oxygen ($^1O_2$), whose electrons are paired, occuring in the same orbital, spins are antiparallel or the electrons occur in different orbitals and also have the opposite spins. These two of a total of five possible excited states of oxygen are known as $^1\Delta$ (delta) singlet oxygen and $^1\sum$ (sigma) singlet oxygen. Energy of $^1\Delta$ singlet oxygen is 93.8 kJ/mol higher and energy of $^1\sum$ singlet oxygen is 157 kJ/mol even higher than the energy of the basic triplet state. The $^1\sum$ singlet oxygen is so reactive that it does not survive relaxation to the ground state, but the $^1\Delta$ singlet oxygen is sufficiently stable (its lifetime is several microseconds) and can therefore react with other molecules also occurring in the singlet state. This singlet oxygen is responsible for most of the reactions of oxygen in the activated state and its reactivity resides in its electrophilic nature. Therefore this excited state is simply called singlet oxygen. It reacts with compounds rich in electrons (such as unsaturated compounds) so that electrons are added to the free molecular orbital. Singlet oxygen arises in chemical reactions, enzymatic reactions, by decomposition of hydroperoxides and in some other ways. In foods, the most important formation pathway is by photochemical reactions with the participation of natural photosensitisers.

[22] Photolytic cleavage of fatty acid molecules in the initial stage of autoxidation with UV exposure is minimal and has no practical significance in comparison with photosensitised oxidation that occurs in the presence of photosensitisers during exposure to visible light. Photosensitisers contain a conjugated system of double bonds that easily absorbs light energy. The photosensitiser absorbs a photon (a quantum of light) and is transferred from the basic singlet state to an unstable singlet excited state. The molecule existing in this state may lose energy obtained in three ways: (i) by internal conversion with the loss of thermal energy (transformation into another excited state), (ii) by light emission (through the process of fluorescence, the state where the emission begins and ends is a singlet state), and, finally, (iii) by conversion into a triplet excited state that is an intermediate in the photosensitised reaction (followed by degradation to a lower energy triplet state and further to the basic singlet state with the emission of light; the process is called phosphorescence, the state where the triplet emission begins and where it ends is a singlet state).

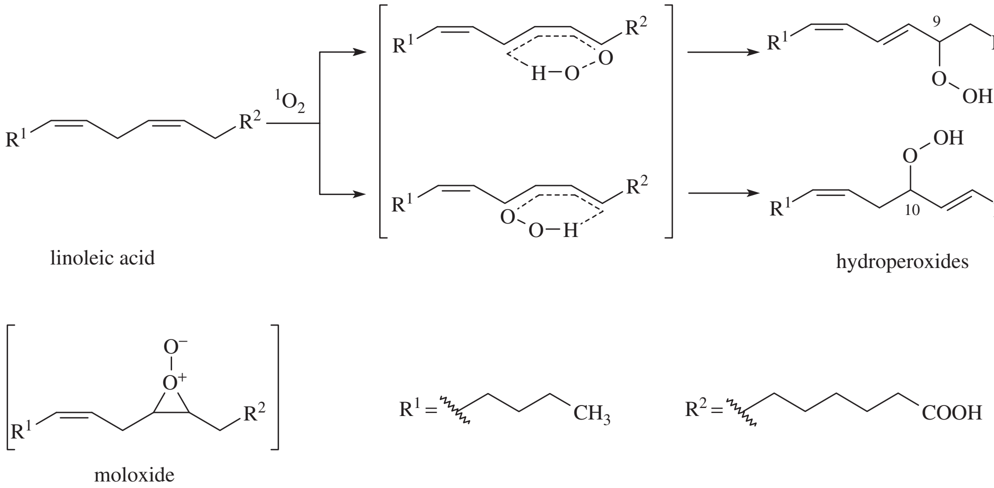


Figure 3.58 Oxidation of unsaturated fatty acids by singlet oxygen.

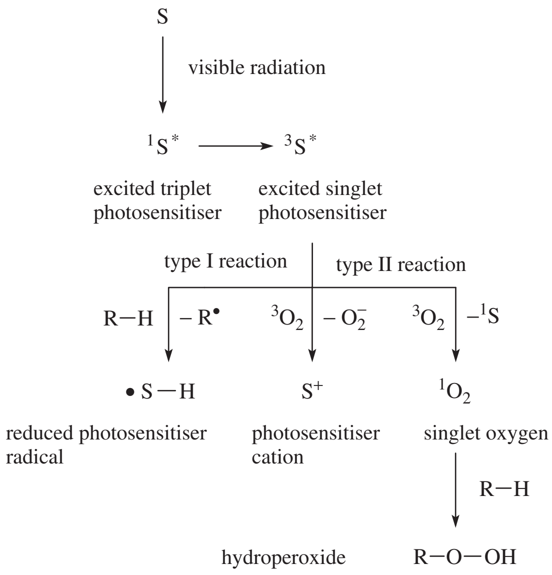


Figure 3.59 Photosensitised oxidation of unsaturated fatty acids.

products, phaeophytins) act mainly as photosensitisers (type II reaction). The concentration of chlorophyll pigments in soybean oil is, for example, 1–1.5 mg/kg; their content in virgin olive oil is higher, around 6 mg/kg. In milk, the main photosensitiser is riboflavin; the main damaged amino acid is methionine. Riboflavin, on absorbing visible light, generates a metastable (triplet) excited state from the initially populated singlet state that may react with triplet molecular oxygen to form singlet oxygen by triplet–triplet annihilation, in the type II photooxidation pathway, or it may react directly with a substrate such as methionine (protein) by accepting hydrogen or electrons and producing radicals through the type I photooxidation mechanism. The significance of the type II reaction in milk has been described in several studies, whereas type I reactions in milk have received less attention. In meat and meat products, myoglobin and oxymyoglobin mainly act as photosensitisers, and the photooxidation product is metmyoglobin.

Other photosensitisers are cofactors of metalloproteins (enzymatic reactions produce, for example, singlet oxygen in some dairy products), some organic compounds such as pyridoxal and its derivatives (see Section 5.10.1), coumarins of the psoralene type (see Section 10.3.2.5.2), polycyclic aromatic hydrocarbons (such as anthracene; see Section 12.2.5), synthetic food colours (e.g. erythrosine; see Section 11.4.1.3) or sulfides, and some metal oxides (such as CdS, ZnS and ZnO).

Scavengers of singlet oxygen

The effects of photosensitisers can be neutralised by substances that serve as scavengers for singlet oxygen. In foods, the most important scavengers are carotenoids, tocopherols and L-ascorbic acid. The mechanism of reactions of carotenoids with singlet oxygen can be simply described by the following reactions:

$$^{1}\text{carotenoid} + {}^{1}O_2 \rightarrow {}^{3}\text{carotenoid} + {}^{3}O_2$$
$$^{1}\text{carotenoid} + {}^{3}S^{*} \rightarrow {}^{3}\text{carotenoid} + S$$
$$^{3}\text{carotenoid} \rightarrow {}^{1}\text{carotenoid}$$

The reaction products of singlet oxygen with carotenoids occurring in the natural singlet state (^{1}carotenoids) are triplet oxygen and carotenoids in the excited triplet state (^{3}carotenoids). Other reaction sequences of carotenoids are described in Section 5.2.6.2.1.

3.8.1.8.5 Enzymatic oxidation

Oxidoreductase enzymes called lipoxygenases (linoleate: O_2 oxidoreductases), formerly known as lipoxidases, are widespread. Multiple forms or isozymes have been detected in both animal and plant species. Lipoxygenases are present in most raw materials and food products, unless they have been heated, as the enzymes are denaturde on heating and lose their activity. Lipoxygenases are non-haem iron-containing dioxygenases involved in the catalysis of the oxidation of polyunsaturated fatty acids containing a (1*Z*,4*Z*)-penta-1,4-diene system of isolated double bonds. They catalyse the oxidation of essential fatty acids to hydroperoxides, but

the other unsaturated fatty acids are not oxidised. Hydroperoxides formed by catalysis of lipoxygenases differ in their optical activity from hydroperoxides formed by autoxidation. Lipoxygenases have different specificity, so they catalyse the formation of hydroperoxide groups only on certain fatty acid carbon atoms and are therefore **regioselective**. Some examples are given in Table 3.46. They are also **stereoselective** as they produce enantiomeric hydroperoxides, while the hydroperoxides arising by autoxidation of fatty acids are racemates. Hydroperoxides of fatty acids produced by the action of lipoxygenases can be further transformed into a variety of biologically active products collectively known as **oxylipins** (oxidised fatty acid derivatives) through the action of several participating enzymes.

The most common substrates for lipoxygenases in plants are linoleic and linolenic acids. Their oxidation, catalysed by lipoxygenases, yields 13- and 9-hydroperoxy fatty acids. Some lipoxygenases mainly catalyse the formation of (13*S*)-hydroperoxides, while less specific lipoxygenases produce a higher proportion of (13*R*)-hydroperoxides. Other lipoxygenases catalyse the formation of optically active 9-hydroperoxides. The 13-hydroperoxides of linoleic and linolenic acids, by the action of hydroperoxide lyases, produce aldehydes with six carbon atoms (Figure 3.60) that possess green (hexanal and hex-3-enal) grassy or beany (nona-3,6-dienal) flavours. Similarly, products with cucumber and melon flavours are formed from 9-hydroperoxides derived from linoleic and linolenic acids (Figure 3.61). For example, isomerisation of (3*Z*,6*Z*)-nona-3,6-dienal yields (2*E*,6*Z*)-nona-2,6-dienal that typically has a flavour resembling fresh cucumbers. Under the action

Table 3.46 Overview of properties of selected plant lipoxygenases.

Plant material	Isoenzyme	Regiospecificity[a]	Occurrence
Soybeans	L-1	95/5	Seeds, water stress
Soybeans	L-2	50/50	Seeds
Soybeans	L-3	50/50	Seeds, water stress
Lentils	Lox1	82/18	Seeds
Potatoes	H1	13-Hydroperoxides	Leaves, wounding
Potatoes	LOX5	9-Hydroperoxides	Tubers

[a]Ratio of 13- and 9-hydroperoxides.

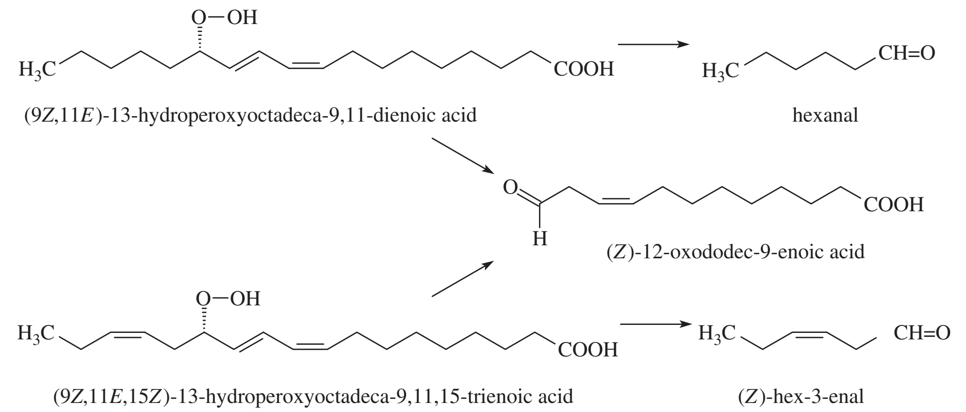


Figure 3.60 Mechanism of action of lyases to 13-hydroperoxides of unsaturated fatty acids.

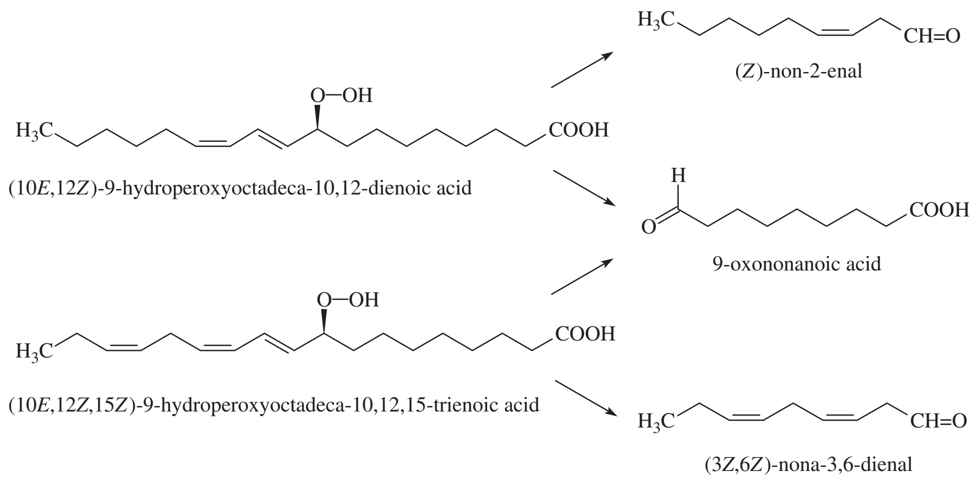


Figure 3.61 Mechanism of action of hydroperoxide lyases to 9-hydroperoxides of fatty acids.

of enal isomerases, *cis*-alkenals are isomerised to *trans*-alkenals or reduced by alcohol dehydrogenases to unsaturated alcohols (Figure 3.62). Both types of reaction products deliver characteristic flavour components to strawberries, bananas, cauliflower, tomatoes and many other fruits and vegetables.

Hydroperoxides of linoleic and linolenic acids also react with sensitive food components, such as carotenes, to form colourless products (lipoxygenase, however, do not directly catalyse the decomposition of carotenes) and induce oxidative changes in sulfur amino acids and proteins. Secondary products of lipid oxidation initiate radical reactions of proteins that lead to a reduction in their nutritional value.

Champignon mushrooms and certain other fungi contain 10-lipoxygenase, which catalyses the formation of unconjugated hydroperoxide, (8*E*,10*S*,12*Z*)-10-hydroperoxyoctadeca-8,12-dienoic acid, from linoleic acid. From this hydroperoxide, lyases produce (*E*)-10-oxodec-8-enoic acid and (*R*)-oct-1-en-3-ol, which is the key component of mushroom aroma (Figure 3.63). Analogously, (8*E*,10*S*,12*Z*,15*Z*)-10-hydroperoxy-8,12,15-octadecatrienoic acid formed by oxidation of linolenic acid yields (3*S*,5*Z*)-octa-1,5-dien-3-ol (Figure 3.63).

Biosynthesis of biologically active oxygenated derivatives of C_{18} fatty acids in plants that are known as **octadecanoids** proceeds by similar mechanisms to the biosynthesis of eicosanoids (such as prostaglandins) in animals. The action of phospholipase A_1, which hydrolyses linolenic acid in the membrane phospholipids and 13-lipoxygenase, which oxidises this acid, yields intermediates such as (9*Z*,11*E*,13*S*,15*Z*)-13-hydroperoxyoctadeca-9,11,15-trienoic acid. This acid then provides, under the action of hydroperoxide dehydratase, (9*Z*,13*S*,15*Z*)-12,13-epoxyoctadeca-9,15-dienoic acid (allene oxide), which yields, with catalysis of allene oxide cyclase, cyclopentanone derivative (10*Z*,15*Z*)-12-oxophyto-10,15-dienoic acid. This acid, through three-stage β-oxidation and reduction by 12-oxophytodienoate reductase, yields the plant

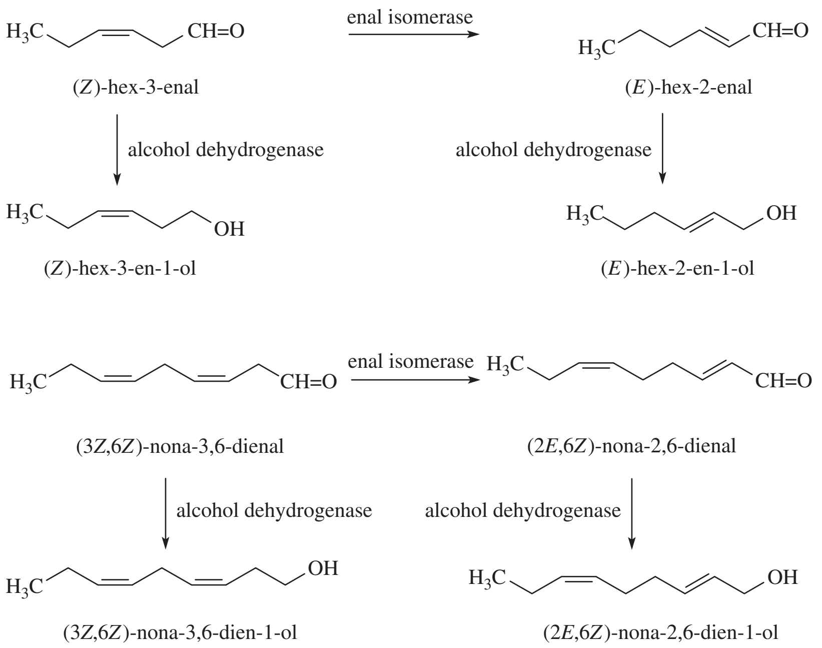


Figure 3.62 Mechanism of action of isomerases and alcohol dehydrogenases to hydroperoxide fission products.

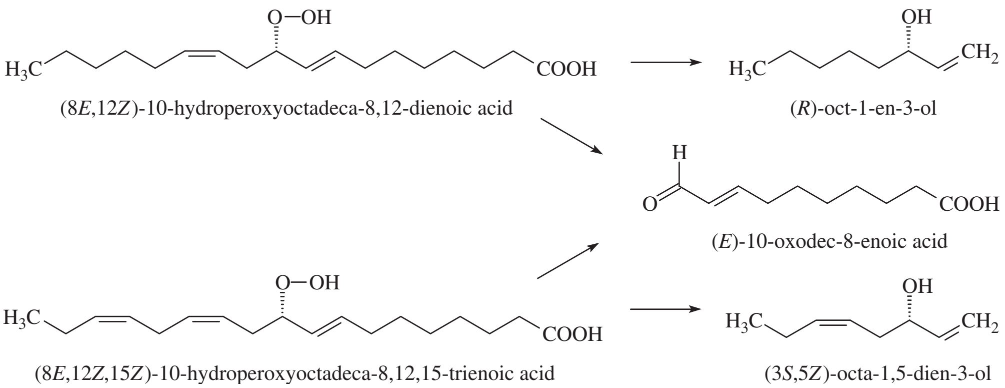


Figure 3.63 Mechanism of action of lyases in mushrooms.

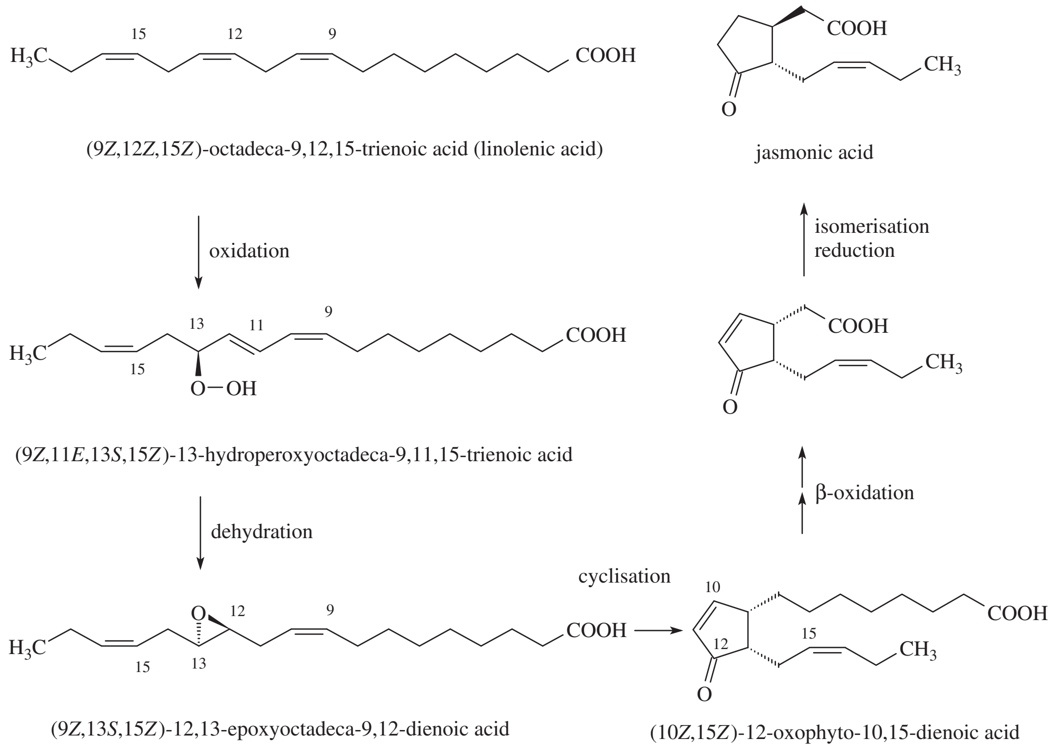
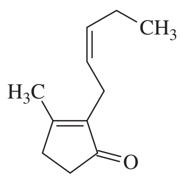


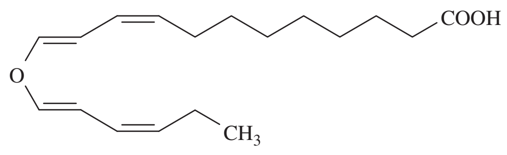
Figure 3.64 Mechanism of action of hydroperoxide cyclases and other enzymes on 13-hydroperoxides of fatty acids.

hormone (−)-jasmonic acid (Figure 3.64). Jasmonic acid plays a role in the inhibition of plant growth and leaf senescence. It is also responsible for the formation of potato tubers and bulbs of some root vegetables, such as onion. Esterification of jasmonic acid, catalysed by carboxyl methylesterase, yields methyl jasmonate. Another volatile transformation product of jasmonic acid is a fragrant ketone (*Z*)-3-methyl-2-(pent-2-en-1-ylcyclopent)-2-en-1-one called *cis*-jasmone (**3-144**). On hydroxylation, *O*-glycosylation and conjugation with amino acids some other metabolites are formed. Volatile jasmonoids occur in the essential oil of jasmine (*Jasminum grandiflorum*, Oleaceae) flowers and in many other essential oils, such as bergamot and lemon essential oils. They are also important components of the aroma of black tea. Jasmonoids are used in plant cells in the regulation of important processes such as seed germination, root growth, fruit ripening and aging, activate defence mechanisms in response to injury, environmental stress factors (e.g. salinity, drought and low temperature) and attack by various pathogens.

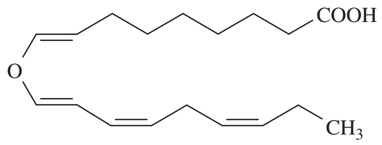


3-144, *cis*-jasmone

Other accompanying enzymes of lipoxygenases are divinylether synthases that catalyse the rearrangement of fatty acid hydroperoxides into divinyl ether fatty acids, which have a role in plant defences towards pathogens. The volatile fission products of these ethers give products with green and grassy flavours. Hydroperoxide generated by 13-lipoxygenases from linolenic acid serves as a precursor of (1*E*,3*Z*,9*Z*,11*E*)-12-hexa-1,3-dien-1-yloxy-9,11-dodecadienoic acid, which is known as etherolenic acid (**3-145**). Similarly, 9-hydroperoxide of linolenic acid is transformed into another divinyl ether fatty acid, (1*E*,3*Z*,6*Z*,8*E*)-9-nona-1,3,6-trien-1-yloxynon-8-enoic acid known as colnelenic acid (**3-146**). Analogously, 13- and 9-hydroperoxides of linoleic acid become precursors of colneleic and etheroleic acids, respectively.



3-145, etherolenic acid



3-146, colnelenic acid

(7*Z*,10*Z*,13*Z*)-Hexadeca-7,10,13-trienoic acid, abundant in many plant species known as 16:3 plants, is a precursor of a range of biological active substances (oxylipins) called **hexadecanoids**.

3.8.1.8.6 *Oxidation catalysed by metals*

Lipid oxidation is catalysed by transition metals that form compounds in many oxidation states, due to the relatively low reactivity of unpaired d electrons. These compounds, which include mainly iron,[23] copper, manganese, nickel, cobalt and chromium, are reduced by adopting one electron. The last three elements are indeed fairly active, but the level of their active forms is so low that these metals are almost of no significance. Other metals, as free ions or some undissociated salts or complexes, act as catalysts directly or indirectly in the initiation, propagation and termination phase of autoxidation reaction.

Initiation reaction

Metals in their higher valency $M^{(n+1)+}$ are initiators of autoxidation reactions. The initiation reaction takes place by electron transfer to form free radicals of hydrocarbons ($R^{\bullet}$), that is, $M^{(n+1)+} + R\text{–}H \rightarrow M^{n+} + R^{\bullet} + H^{+}$, and with ferric ions as $Fe^{3+} + R\text{–}H \rightarrow Fe^{2+} + R^{\bullet} + H^{+}$. The initiation phase is also indirectly catalysed by metals in the lower valency M^{n+} via their transient metal complexes with oxygen. The reaction products are hydrocarbon radicals ($R^{\bullet}$), metals in higher valences, reactive oxygen species such as superoxide radical anion ($O_2^{-\bullet}$) or the protonised form of the superoxide radical ($HO_2^{\bullet}$):

$$M^{n+} + O_2 \rightarrow [M^{(n+1)+} \ldots O_2] \xrightarrow{R-H} R^{\bullet} + [M^{(n+1)+} \ldots {}^{-}O_2H]$$

$$M^{n+} + O_2 \rightarrow [M^{(n+1)+} \ldots O_2] \xrightarrow{R-H} R^{\bullet} + M^{n+} + HO_2^{\bullet}$$

$$M^{n+} + O_2 \rightarrow [M^{(n+1)+} \ldots O_2] \xrightarrow{R-H} RO^{\bullet} + [M^{(n+1)+} \ldots {}^{-}OH]$$

$$M^{n+} + O_2 \rightarrow [M^{(n+1)+} \ldots O_2] \xrightarrow{(M^{n+} \cdots R-H)} HO_2^{\bullet} + [M^{(n+1)+}R^{-}] + M^{n+}$$

$$M^{n+} + O_2 \rightarrow [M^{(n+1)+} \ldots O_2] \rightarrow M^{(n+1)+} + O_2^{-\bullet}$$

The autoxidation reaction is also indirectly catalysed by reactive oxygen species (e.g. $O_2^{-\bullet}$, $HO_2^{\bullet}$ and H_2O_2), which form by the above mentioned and other reactions:

$$O_2^{-\bullet} + H^{+} \rightarrow HO_2^{\bullet}$$

$$O_2^{-\bullet} + H^{+} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2$$

The reaction of superoxide radical $HO_2^{\bullet}$ with unsaturated acids is very slow and, therefore, is not too significant. Superoxide radical anion $O_2^{-\bullet}$ does not react with fatty acids. However, much more reactive are hydroxyl radicals generated by reduction of hydrogen peroxide by metals that directly initiate autoxidation reaction. As well as metals, these radicals are one-electron initiation agents:

$$M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + HO^{\bullet} + HO^{-}$$

$$R\text{–}H + HO^{\bullet} \rightarrow R^{\bullet} + H_2O$$

Propagation reactions

Some metals (Fe, Cu and Ni) catalyse the decomposition of hydroperoxides to alkoxyl radicals in their lower valency, which results in a change to a higher valency. Metals in the higher valency then catalyse the decomposition of hydroperoxides to peroxyl radicals and switch to a lower valency:

$$R\text{–}O\text{–}OH + M^{n+} \rightarrow R\text{–}O^{\bullet} + HO^{\bullet} + M^{(n+1)+}$$

$$R\text{–}O\text{–}OH + M^{(n+1)+} \rightarrow R\text{–}O\text{–}O^{\bullet} + H^{+} + M^{n+}$$

The resulting alkoxyl and peroxyl radicals increase the autoxidation reaction rates of initiation and propagation phases, since the rate of cleavage of hydroperoxides by metal ions is much faster than the formation of radicals *ab inicio*. Metal ions and non-ionised salts may react in this way (Figure 3.65). Of the metals bound in complexes, some are effective, but some are ineffective. Metals may also become less effective in the presence of fats if micelles are formed. The catalyst for the oxidation of lipids may be bound iron complexes. Iron bound in haem pigments has the same catalytic activity as the ions Fe^{2+} a Fe^{3+}, in aqueous solutions it is even more active, as it catalyses the cleavage of hydroperoxides as follows:

$$R\text{–}O\text{–}OH + Fe^{2+} \rightarrow R\text{–}O^{\bullet} + HO^{-} + Fe^{3+}$$

$$R\text{–}O\text{–}OH + Fe^{3+} \rightarrow R\text{–}O\text{–}O^{\bullet} + H^{+} + Fe^{2+}$$

In addition to these reactions, resulting in direct electron transfer, four other mechanisms have been postulated that may be involved in catalysis of lipid oxidation by haem iron. The first of these mechanisms implies formation of complexes with hypervalent iron (with valency +4), which can oxidise lipids directly. The second mechanism is indirect. Haem iron catalyses the formation of hydroxyl radicals $HO^{\bullet}$, which (as one electron agents) initiate the autoxidation reaction. Hydroxyl radicals are formed by reaction of Fe^{2+} ions bound in haem pigments via $O_2^{\bullet-}$ and hydrogen peroxide (H_2O_2), which is reduced to hydroxyl radicals with the formation of haematin:

$$H_2O_2 + \text{haem} - Fe^{2+} \rightarrow HO^{\bullet} + HO^{-} + \text{haem} - Fe^{3+}$$

The third mechanism of catalysis of lipid autoxidation by haem pigments is a photosensitised reaction and oxidation of lipids by either singlet oxygen or free radicals. The fourth mechanism assumes hydroxyl radical attack, in which haem iron is released in an ionic form, which then catalyses the oxidation of lipids in the same way as non-haem iron.

The redox potentials of other metals, such as Mn, Co and so on, are too low, therefore these metals cannot catalyse the cleavage of hydroperoxides in aqueous systems (e.g. in emulsions), but can

[23] It is not yet quite clear whether, in addition to ferric (Fe^{3+}) and ferrous ions (Fe^{2+}), whether mixed complexes of both ions with oxygen ($Fe^{3+}\text{–}O_2\text{–}Fe^{2+}$) and the so-called hypervalent iron (ions with valences +4 or + 6, for example ferryl cations FeO^{2+} and ferrate anions FeO_4^{2-}), which are the active forms of the enzymes containing haem cofactors (such as peroxidases, catalases and cytochrome P-450) are also involved in the process of the oxidation of lipids.

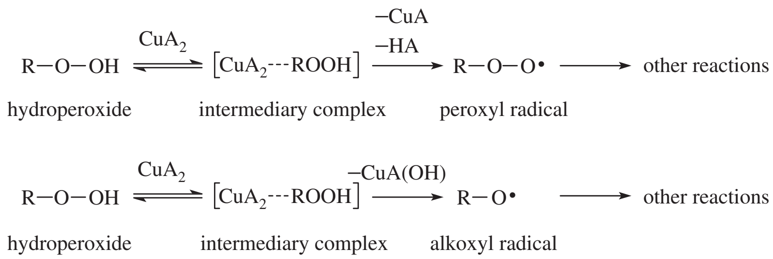


Figure 3.65 Catalytic activities of cuprous salts in autoxidation reactions. CuA_2 = cuprous salt, CuA = cupric salt, HA = fatty acid, CuA(OH) = hydrated cupric salt.

catalyse the decomposition of lipid hydroperoxides in anhydrous fats through the transient complexes formed with hydroperoxides:

$$\text{R–O–OH} + Co^{2+} \rightarrow [\text{R–O–OH} \ldots Co]^{2+}$$
$$\rightarrow \text{R–O}^{\bullet} + Co^{3+} + HO^{-}$$
$$\text{R–O–OH} + Co^{3+} \rightarrow [\text{R–O–OH} \ldots Co]^{3+}$$
$$\rightarrow \text{R–O–O}^{\bullet} + Co^{2+} + H^{+}$$

Inhibition

Since the effects of heavy metals increase the amount of free radicals in the lipid phase, not only do the rates of initiation and propagation reactions increase, but also the rate of termination reaction increases. Heavy metals therefore also change the composition of the reaction products. At high concentrations of free radicals, the termination reaction may dominate and metals then act as the inhibitors of autoxidation. Autoxidation reaction can also be inhibited by metals when they are present at higher concentrations. It is assumed that the reason is the oxidation and reduction of free hydrocarbon radicals to anions and cations by ions of Fe and Cu and the formation of complexes of free radicals. Other complexes are also formed with Co. All these reactions interrupt the radical chain autoxidation reaction. Reactions with Fe ions are given as examples.

$$R^{\bullet} + Fe^{2+} \rightarrow Fe^{3+} + R^{-}$$
$$R^{\bullet} + Fe^{3+} \rightarrow Fe^{2+} + R^{+} \rightarrow \text{products}$$
$$R^{\bullet} + Fe^{3+} \rightarrow [R^{\bullet} \ldots Fe^{3+}]$$

The formation of Co complex can be described by the following equations:

$$R^{\bullet} + CoA_3 \rightarrow \text{R–}CoA_2$$
$$\text{R–O}^{\bullet} + CoA_3 \rightarrow \text{R–O–}CoA_2$$
$$\text{R–O–O}^{\bullet} + CoA_3 \rightarrow \text{R–O–O–}CoA_2$$
$$(CoA_3 = \text{undissociated salt of } Co^{3+} \text{ with fatty acids})$$

Crude oil contains mainly iron and copper at levels up to several milligrams per kilogram, but during degumming and subsequent refining the content of these metals is reduced to negligible amounts. In foods, however, these metals are present in considerable concentrations and can pass into the oil phase, although the original oil may have contained only traces of the metals.

3.8.1.9 Inhibitors of oxidation

The inhibitors of oxidation reaction of fats are substances which reduce the oxidation rate, regardless of the mechanism of their action. These compounds include antioxidants, synergists, chelating agents and compounds decomposing hydroperoxides by non-radical reactions. Also agents stabilising hydroperoxides may reduce the reaction rate because they inhibit the formation of free radicals.

3.8.1.9.1 Antioxidants

Antioxidants (see Section 11.2.2) are substances that can react with free radicals of the autoxidation chain, especially with peroxyl radicals (Figure 3.66). The reaction creates hydroperoxides or other non-radical lipid products. The antioxidant is transformed to the form of a free radical, which, however, is fairly stable, so it is unable to continue in the autoxidation reaction. The role of the antioxidant thus lies in shortening the autoxidation chain and increasing the rate of termination reactions. During the reaction the antioxidant is consumed. When all of the antioxidant has been consumed, the autoxidation reaction proceeds as if no antioxidant was present. Antioxidants therefore cannot completely stop the autoxidation reaction; they just slow this reaction down, ideally to the initial reaction rate.

Figure 3.67 shows the reaction without an antioxidant and the reaction in its presence. It is clear that antioxidants prolong the induction period (slow autoxidation), but do not affect the rate of the subsequent rapid oxidation. The ratio of the length of the induction period of inhibited and uninhibited reactions is called the **protection factor**. It is usually expressed as a percentage of increased stability (extension of the induction period).

In addition to the inhibition reaction, other reactions run in parallel, such as oxidation of free antioxidant radical to peroxyl

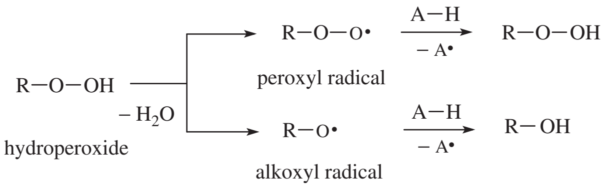


Figure 3.66 Reactions of antioxidants with free radicals formed by autoxidation of fatty acids: A–H = antioxidant, A• = antioxidant radical.

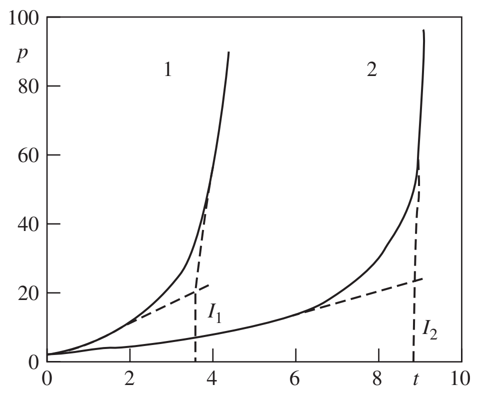


Figure 3.67 Impact of added antioxidant on the course of autoxidation reactions: p = amount of fatty acid hydroperoxides in milliequivalents of active oxygen per kg (peroxide number), t = duration of autoxidation in days, 1 = antioxidant (BHA) concentration = 0%, 2 = 0.02%, I_1 and I_2 = corresponding induction periods, protection factor $PF = (I_2 - I_1)/I_1$.

radical or the reverse reaction with the formation of a free lipid radical, especially at high antioxidant concentrations. The reaction mechanism is given in detail in Section 11.2.2.2.

The most commonly used antioxidants are synthetic phenol derivatives, which contain two (or three) hydroxyl groups in the *ortho*- or *para*-positions. Natural antioxidants are usually substituted in the *ortho*-position, so they are more efficient as antioxidants. Synthetic antioxidants are usually substituted in the *para*-position, so they are less toxic than the corresponding *ortho*-derivatives. Instead of one hydroxyl group, a methoxyl group or at least a branched alkyl substituent may be present. Substitution in a benzene ring by additional alkyl group or two alkyls increases the antioxidant efficiency. Far less frequently used antioxidants, especially for their higher toxicity, are compounds with heterocyclic nitrogen (such as dihydropyridine or dihydroquinoline derivatives). Natural antioxidants, mainly tocopherols (see Section 5.4.1), occur in all natural fats and oils. Other common natural antioxidants include phenolic and numerous other compounds (see Section 11.2.2.4).

3.8.1.9.2 Synergists and sequestrants

Synergists are substances that do not have antioxidant activity themselves, but are able to increase the efficiency of antioxidants. The most common synergists include polyhydric acids, such as citric, tartaric, malic, ascorbic and phosphoric acids. These substances are not very soluble in fats, which limits their activity. Therefore, their lipophilic derivatives, such as esters of ascorbic acid (see 5-106) or phospholipids instead of phosphoric acid, are often used. The mechanism of synergism is not fully elucidated and is probably not uniform. It may be caused to a certain extent by the non-radical acceleration of decomposition of lipid hydroperoxides, partly by the regeneration of antioxidants, and partly by the binding of prooxidants (mainly heavy metals) into inactive complexes. This last mechanism is fairly widespread and the synergist is also a chelating agent or sequestrant, a chemical that promotes sequestration. Numerous substances have the ability to bind heavy metals to complexes, such as pyrocatechol and pyrogallol derivatives, some carbohydrates, acidic phospholipids and lysophospholipids, oxalic acid, phytic acid and other compounds. Some sequestrants are used as food additives, for example ethylenediaminetetraacetic acid (EDTA). Metal ions are inactivated due to formation of insoluble and undissociated salts, but also some soluble complexes may be inactive, while others may act as prooxidants.

3.8.1.9.3 Heterolytic decomposition of hydroperoxides

Lipid hydroperoxides mostly decompose into free radicals, but sometimes they can decompose into ions, for example in the presence of acids and alkalis (Figure 3.68), or by reactions with transition metals.

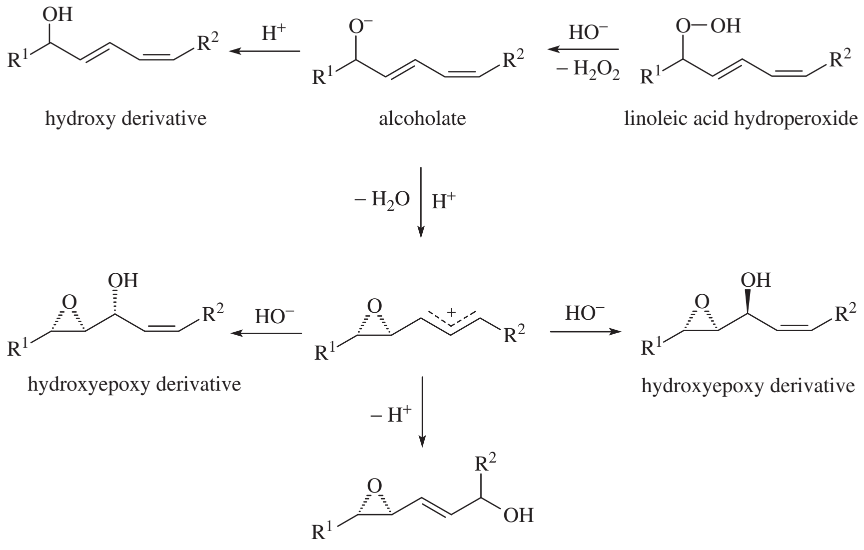


Figure 3.68 Heterolytic decomposition of hydroperoxides in acid and alkaline media.

3.8.1.10 Oxidation and quality of lipids

3.8.1.10.1 Rancidity during storage

Oxidation reactions that proceed during storage of fats reduce their sensory quality. Such processes are known as rancidity. Rancidity is not just caused by oxidation of fatty acids, but also by other reactions. Therefore, several types of rancidity can be distinguished:

- **hydrolytic rancidity**
- **oxidative rancidity**
- **ketonic rancidity**
- **flavour reversion.**

Hydrolytic rancidity

Fatty acids released during hydrolysis of fats do not bring about fat rancidity, because they are sensorially imperceptible in small amounts. The exceptions are fats containing bound fatty acids with shorter carbon chain (4–10 carbons). Butyric acid is released from butter and imparts a nasty, very pungent odour. Acids with 6–10 carbon atoms are also present to a larege extent in coconut or palm kernel oils, as well as in milk fats. Native enzymes or enzymes released by molds give the product a typical soapy off-taste.

Oxidative rancidity

Hydroperoxides resulting in fat oxidation do not affect the sensory quality of fats and oils, but their decomposition products produce distinctive odours that depend on the concentration of the secondary products and their composition. These reactions are described in detail in other sections. A certain range of oxidation reactions, both enzymatic and non-enzymatic, is often desirable, because it leads to the formation of characteristic flavour active products in many foods (such as fruits, vegetables and fried foods).

Ketonic rancidity

Ketonic or perfume rancidity typically occurs in butter, where it is undesirable. Fatty acids with 6–12 carbon atoms released from triacylglycerols by hydrolysis with lipases of microorganisms (e.g. of fungi of the genera *Penicillium* and *Aspergillus*) are enzymatically degraded largely by β-oxidation[24] to 3-oxoacids. Their decarboxylation yields alkan-2-ones, known as methylketones, which can be reduced to the corresponding alkan-2-ols (Figure 3.69). The most common methylketones (see Section 8.2.4.1.2) are pentan-2-one, hexan-2-one, heptan-2-one, octan-2-one and nonane-2-one that have specific perfume odours. Pentane-2-one and hexane-2-one posses fruit odour reminiscent of bananas, heptan-2-one has a floral and herbal odour, octane-2-one has a flowery odour and nonan-2-one exhibits a floral and oily odour. The odour itself is not unpleasant, and in mold cheeses it is even desirable, but is unusual and atypical in edible fats.

Flavour reversion

Flavour reversion is characteristic of soybean oil, and sometimes also occurs in other oils containing linolenic acid, for example rapeseed oil. It manifests itself when the oil still contains fairly low amounts of fatty acid hydroperoxides. Smells resembling grass and beans are due to flavour reversion, and are caused by different compounds resulting from the decomposition of hydroperoxides, including various derivatives of furan. Oil, in which this defect is apparent, can have this smell removed during refining, but the defect re-appears after a certain amount of time (hence the name

[24] Acyl-CoA synthetase synthesises, in the presence of HS-CoA, acyl-CoA from a saturated fatty acid. Acyl-CoA is transformed by acyl-CoA dehydrogenase into 2,3-dehydroacyl-CoA, and isomerism leads to *trans*-2-enoyl-CoA, enoyl-CoA hydratase yields (*S*)-3-hydroxyacyl-CoA, which is transformed into 3-oxoacyl-CoA by 3-hydroxyacyl dehydrogenase; the latter compound is hydrolysed by 3-ketoacyl-CoA thiolase to HS-CoA and 3-oxoacid. This acid eliminates carbon dioxide and yields methylketone, with catalysis of decarboxylase. Reductase can reduce methylketone to a secondary alcohol.

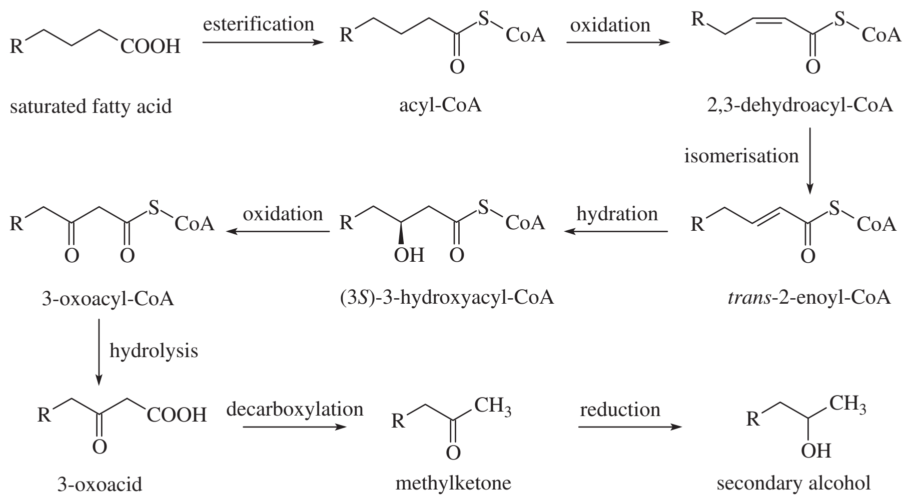


Figure 3.69 Formation of alkan-2-ones in ketone rancidity.

flavour reversion). Flavour reversion is especially off-putting to consumers in the United States as they require utterly tasteless cooking oil. Substances causing flavour reversion are thus actually present in small quantities that many other consumers do not mind, provided they ever notice the flavour change.

3.8.1.10.2 Changes during frying

During frying, the oil or fat is preheated to temperatures around 150–200 °C. The frying process is commonly carried out in:

- a thin layer of fat
- a layer of fat more than 50 mm deep (usually between 100 and 200 mm) where the fried food is submerged in fat or floats on fat.

The second approach is more common today and is called **deep frying** or **deep fat frying**. After inserting the food, water evaporates upon contact with hot fat, which cools the fat. During the deep frying process, which usually takes a few minutes, the temperature again increases by a few tens of degrees. In the processes taking place in the fat exposed to high temperature in the presence of air and moisture, several types of reactions can be distinguished:

- Hydrolytic processes induced by hot water vapour (released from the fried food) action on the hot fat are often the main processes occurring during frying; they lead to the hydrolysis of fatty acids, which are largely adsorbed on fried food or escape into the air.
- Oxidation processes that are very fast at frying temperatures; dissolved oxygen in fat is consumed, so that further oxidation is slow and depends on the rate at which oxygen diffuses from the air; the rate of diffusion, however, significantly increases if oil starts to foam, as foaming increases the interface between fat and air.
- Processes causing both polymerisation reactions between free radicals and reactions of carboxyl groups of free fatty acids with hydroxyl and epoxy groups of oxidised fatty acids of fat.
- Pyrolytic processes such as dehydration of oxidation products or their reactions with proteins and other components of fried foods; they yield significant sensorially active substances; pyrolytic reactions include the decomposition of glycerol (released by hydrolytic processes) to acrolein (see Section 8.2.4.1.1).

Under conditions employed in deep frying, a complex series of reactions takes place resulting in a loss of quality of both the frying oil and the fried food. The levels of polar compounds, polymers, free fatty acids, oxidised fatty acids and the smoke point of the fat have become the most generally accepted indicators for quality evaluation of frying fats and are included in some of the current official regulations. In general, if the content of polar compounds generated by oxidative and hydrolytic reactions exceeds 25% and the amount of polymers exceeds 10% of the fat weight, it is recommended that the used fat be replaced with fresh fat.

3.8.1.11 Effects of oxidised lipids on human health

Oxidised lipids have only a low acute toxicity; therefore their effect on human health is often underestimated. The toxicity of fats used for frying has not been firmly established, therefore the limits for their use (25% of polar compounds and 10% of polymers) indicate that in reality the functional properties are deteriorating and the flavour of the fried food will get worse. Adversely reflected in terms of chronic toxicity are cyclic dimers and particularly cyclohexene derivatives. A higher content of hydroperoxides causes symptoms of vitamin E and essential fatty acid deficiency, which results in increased permeability of the skin to water. It is also difficult to enzymatically hydrolyse (digest) oxidised lipids. Recently it has been shown that a higher intake of oxidised fats increases their levels in blood serum, and oxidised fatty acids or free radicals arising from oxidised fatty acids then react with certain proteins in blood serum and in the walls of blood vessels and form atherosclerotic deposits. Oxidised sterols are particularly active in this respect. Similar deposits are formed, for example, in the nerve tissue and some other important organs. Oxidation products of lipids, especially the reactive acrolein, (*E*)-4-hydroxyalk-2-enals and malondialdehyde, also react with proteins and nucleic acids, where any alterations may facilitate development of malignant tumours. For these reasons, an increased intake of natural antioxidants, mainly tocopherols and carotenes, is recommended, which leads to an increased intake of easily oxidisable polyenoic fatty acids.

3.8.1.12 Oxidation of food constituents and other reaction of oxidised lipids

Oxidation products of lipids (hydroperoxides, free alkoxyl and peroxyl radicals, epoxides and aldehydes) react with a number of food constituents during the processing and storage of food. These reactions often lead to a reduction in the nutritional value of foods (such as reactions with proteins and vitamins) and a deterioration of their organoleptic properties (e.g. reactions with flavour active substances).

3.8.1.12.1 Reactions with proteins and deoxyribonucleic acids

Reactions of proteins with lipid hydroperoxides and other oxidised lipids give rise to different types of lipoproteins, in which lipids and proteins are bound by physical bonds (as in natural lipoproteins), but also by covalent bonds. Reactions with hydroperoxides, free radicals, epoxides and aldehydes lead to the fission of some protein bonds, formation of protein radicals and oligomers, cross links between protein chains and some sensitive functional groups of amino acids are oxidised (see Section 2.5.1.1).

Cysteine and methionine are particularly sensitive to oxidation, as are histidine, arginine, tryptophan and phenylalanine. In foods, oxidation mainly takes place in sulfur-containing amino acids (cysteine, cystine and methionine). Cysteine can be oxidised to cystine or even to cysteic acid, but the oxidation by hydroperoxides in foods does not go so far. Methionine is oxidised to

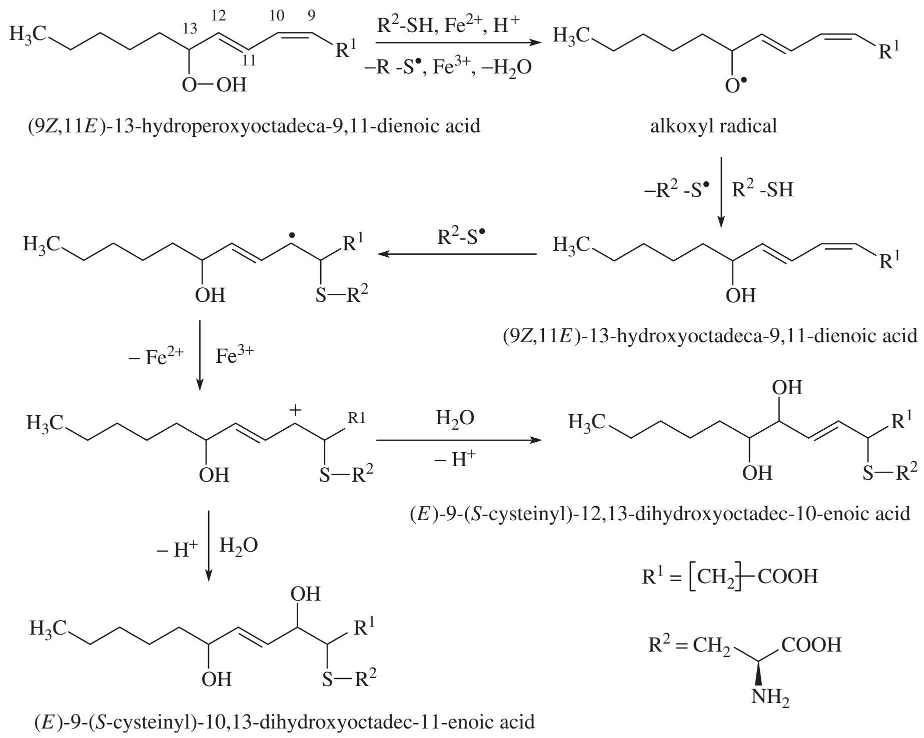


Figure 3.70 Reaction of cysteine with 13-hydroperoxyoctadeca-9,11-dienoic acid.

methionine sulfoxide, but not to methionine sulfone. Figure 3.70 gives as an example of a simplified reaction of cysteine with 13-hydroperoxyoctadeca-9,11-dienoic acid, which is formed by oxidation of linoleic acid. Further reactions of sulfur-containing amino acids are presented in the section dealing with the reactions of amino acids (see Section 2.5.1.1). Also sensitive to oxidation are thiols, sulfides, oligosulfides and inorganic sulfides. Selenium compounds are oxidised in the same way as sulfides. Furthermore, selenites can be oxidised to selenates.

The reaction of hydroperoxides with primary amino groups probably produces an imine, according to the mechanism indicated in Figure 3.71. Imines are also formed as reaction products of protein-bound amino acids with carbonyl compounds resulting as secondary products of lipid oxidation. In particular, 2-alkenals (α,β-unsaturated aldehydes), such as acrylamide, (*E*)-4-hydroxyhex-2-enal, (*E*)-4-hydroxynon-2-enal and (*E*)-4,5-epoxydec-2-enal and malondialdehyde, are very reactive compounds that accumulate in forms covalently bound to proteins and nucleosides *in vivo* and can also be found in foods. The covalent products of these aldehydes, known by the acronym **ALE** (advanced lipoxidation end products) are used as markers of oxidative stress in the organism. Analogous reaction products of proteins with reactive products arising from sugars, known as AGEs, are described in Section 4.7.5.6. Dietary ALEs derived from acrolein and other alk-2-enals are found in the greatest amounts in heat-treated dairy and meat products.

Of all the 2-alkenals, acrolein is by far the strongest electrophile and, therefore, shows the highest reactivity with nucleophiles, such as the sulfhydryl group of cysteine, ε-amino group of lysine and imidazole group of histidine. Acrolein is present

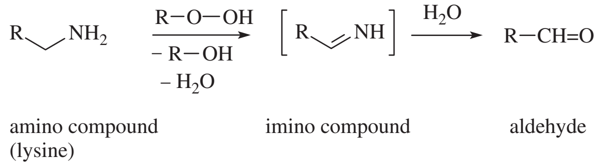


Figure 3.71 Reaction of hydroperoxides with amines or basic amino acids.

in foods mainly as a decomposition product of methionine (see Figure 2.61), arachidonic acid (see Figure 3.72), glycerol (see Section 8.2.4.1.1), hydroxyacetone (acetol, Figure 4.60) and spermine (see Section 10.3.2.10.3). With the amino group of amino acids, acrolein undergoes nucleophilic addition at the double bond (C-3) to form a derivative with retention of the aldehyde group, resulting in the formation of the Michael addition-type acrolein–amino acid adducts. It has been shown that acrolein modifies lysine and histidine residues of human serum albumin and some proteinases. Although it has been proposed that, upon reaction with amino groups, acrolein forms β-substituted propanals (R–NH–CH_2–CH_2–CH=O) and imine (R–NH–CH_2–CH_2–CH=N–R), an aduct of acrolein (two molecules) with lysine, 2-amino-6-(3-formyl-1,2,5,6-tetrahydropiperidino) hexanoic acid, also known as N^{ε}-(3-formyl-3,4-dehydropiperidino) lysine or FDP-lysine (**3-147**), has recently been identified as the major product.

(*E*)-4-Hydroxyalk-2-enals arising from lipid peroxidation in biological membranes *in vivo* and in foods have been proven

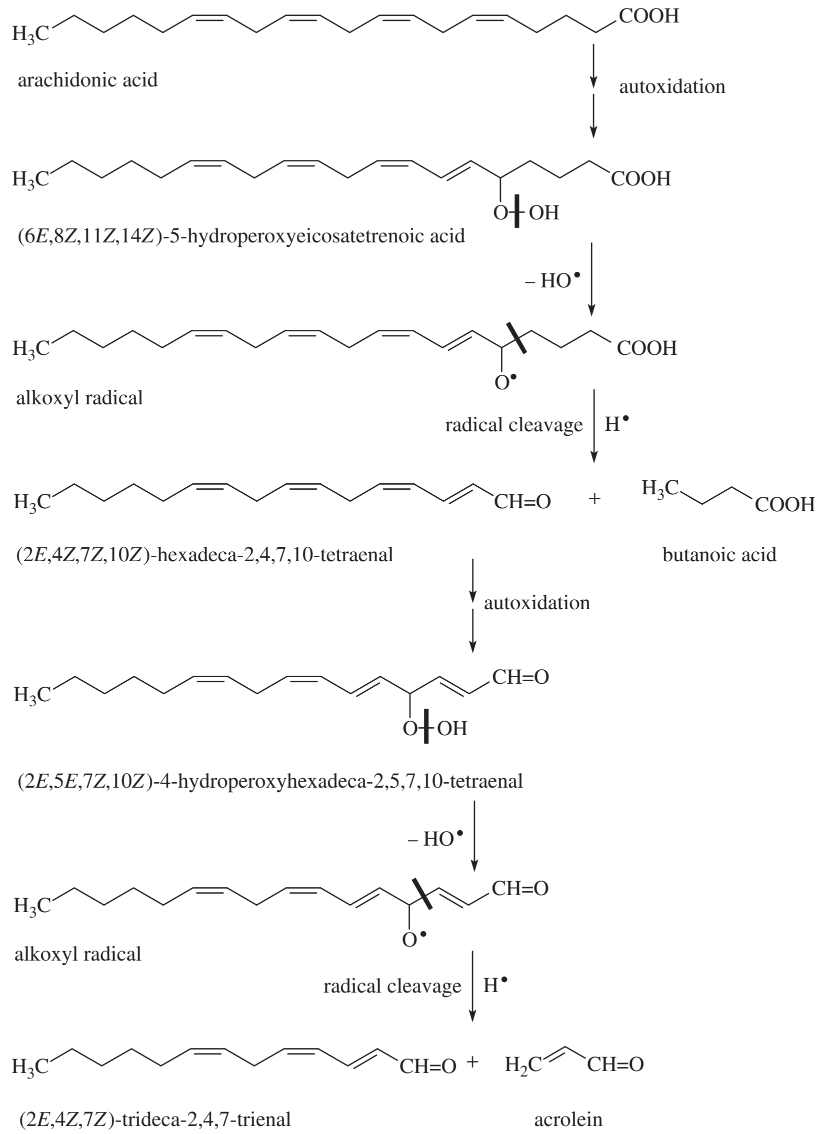
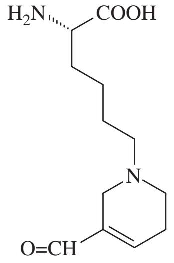


Figure 3.72 Formation of acrolein from arachidonic acid.



3-147, 2-amino-6-(3-formyl-1,2,5,6-tetrahydropiperidino)hexanoic acid

to be involved in carcinogenesis. The most important compound is (*E*)-4-hydroxynon-2-enal, which arises from the ω-6 fatty acids (linoleic or arachidonic acids) (Figure 3.73). Linolenic acid analogously yields (*E*)-4-hydroxyhex-2-enal. 4-Hydroxynon-2-enal has also been found in many animal tissues, where it is produced in significant quantities during oxidative stress. In foods, it is formed in various meat products, such as frankfurter suasages (0.08–0.62 mg/kg), and in cooking oils used repeatedly in catering and in households. For example, the thermally oxidised soybean oil after intermittent heating at 185 °C for 1 h contained 2.3 mg/kg of 4-hydroxynon-2-enal. 4-Hydroxynon-2-enal has various beneficial effects (such as stimulation of guanylate cyclase and phospholipase C) at low concentrations (below 0.1 mM), whereas at higher concentrations (1–20 mM) it inhibits protein and DNA syntheses, activates phospholipase A2 and is associated with many diseases, such as chronic inflammation, neurodegenerative diseases, atherosclerosis, diabetes and various types of cancer. Detoxification and elimination of 4-hydroxynon-2-enal is provided by several enzymes, including glutathione *S*-alkyltransferase, also known as *S*-hydroxyalkyl glutathione lyase (which catalyses the conjugation of aldehyde to glutathione with the formation of polar products) and aldehyde dehydrogenase (which reduces

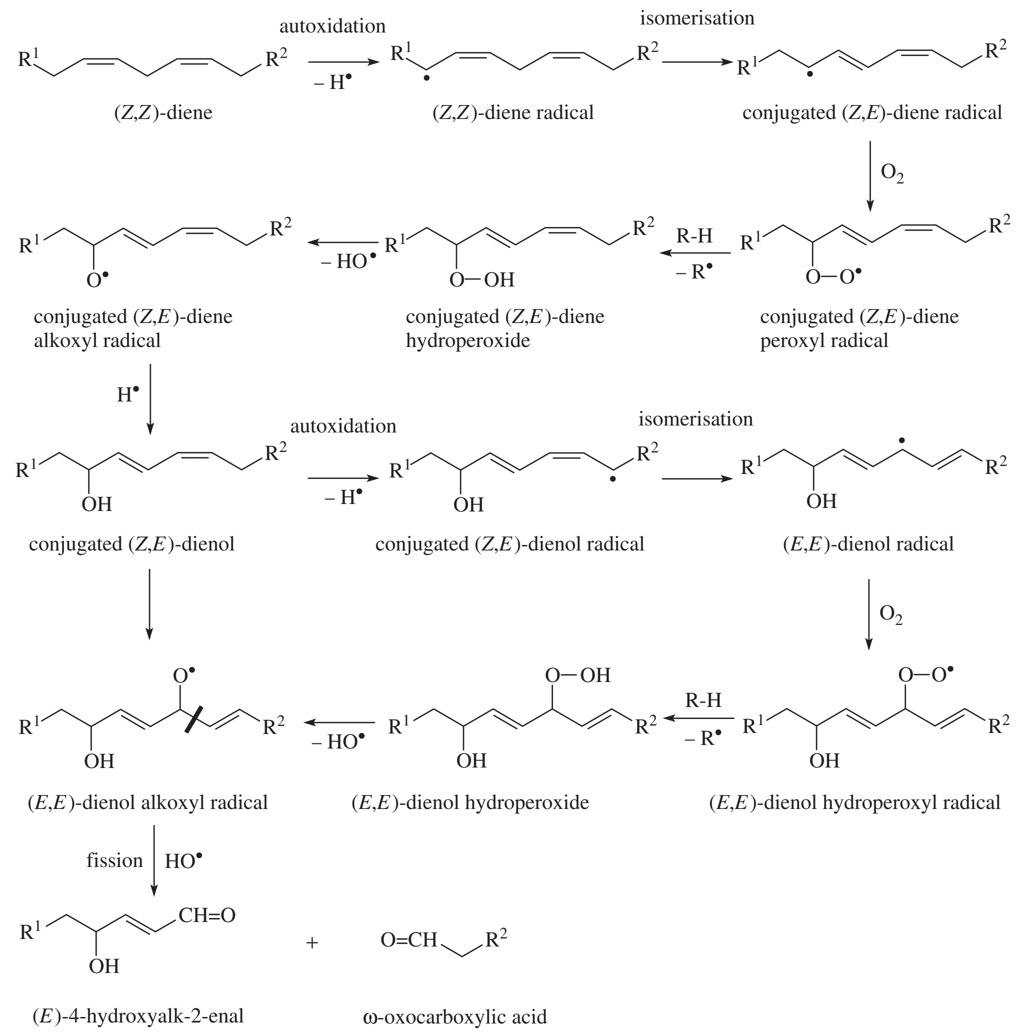
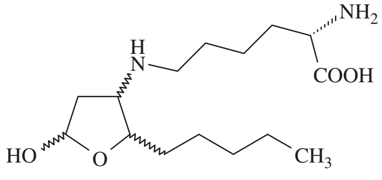
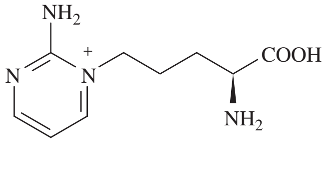


Figure 3.73 Expected mechanisms of formation of (*E*)-4-hydroxyalk-2-enals from ω-6 fatty acids.

aldehyde to alcohol). An example of a product of 4-hydroxynon-2-enal with lysine is 3-(N^{ε}-lysino)-4-hydroxynonan-1-ol, known as HNE-lysine (**3-148**), while a 2-aminopyrimidine derivative, HNE-arginine (**3-149**), results from a reaction with arginine. Analogous products arise in reactions of 4-hydroxynon-2-enal with cysteine and histidine.



3-148, HNE-lysine



3-149, HNE-arginine

Considerable attention has also been paid to malondialdehyde, but the mechanisms of its formation from fatty acids are not yet fully understood. As shown in Figure 3.51 and Figure 3.53, malondialdehyde arises as a secondary oxidative product by cleavage of alk-2-enals and alka-2,4-dienals. Its precursors are thus, under certain conditions (acidic pH, singlet oxygen), dienoic fatty acids, such as linoleic acid. Biochemically, the most important sources of malondialdehyde, however, are probably cyclic peroxides of fatty acids with three or more double bonds and similar structures (hydroperoxybisepidioxides, hydroperoxybiscycloendoperoxides and dihydroperoxides) (Figure 3.36). Malondialdehyde in tissues may also arise from certain precursors of prostaglandins. For example, (9*Z*,13*E*,15*Z*)-12-hydroperoxy-9,13,15-octadecatrienoic acid (12-hydroperoxylinolenic acid) formed by oxidation of linolenic acid can cyclise to a derivative of 1,2-oxolane, oxidation of which yields a cyclic peroxohydroperoxide as a major product. Cleavage of bonds on both sides of peroxohydroperoxides gives malondialdehyde (Figure 3.74). This aldehyde readily reacts with functional groups

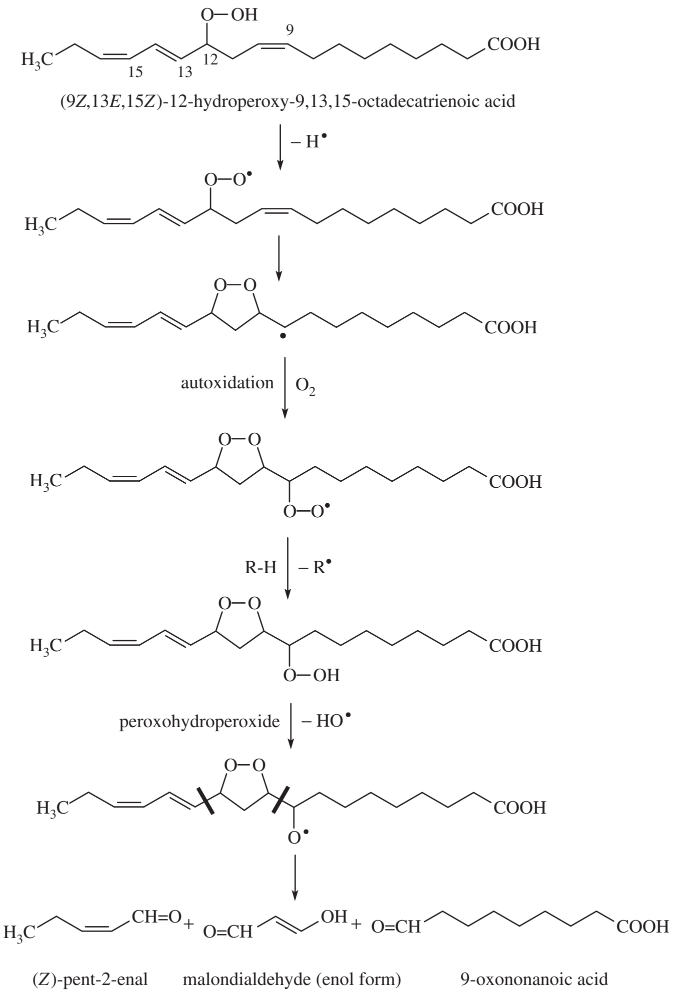
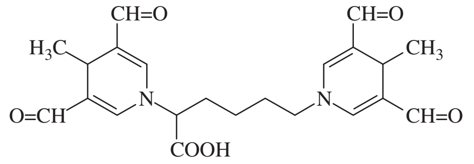


Figure 3.74 Formation of malondialdehyde from linolenic acid peroxohydroperoxide.

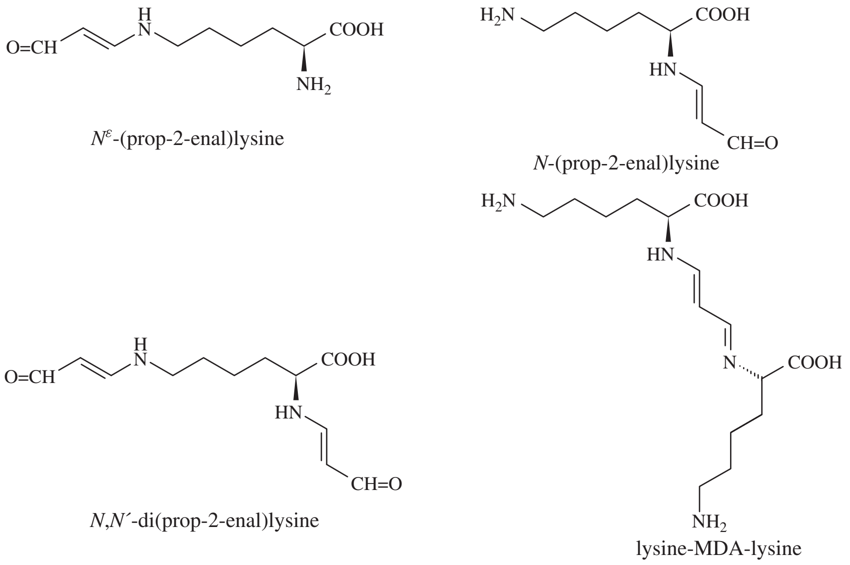
present in proteins, nucleic acids and phospholipids, especially amino groups.

Reaction of a malondialdehyde (MDA) carbonyl group with amino groups leads to the formation of imines, but formation of these structures is of no nutritional concern, because they are hydrolysed at the acidic pH of the stomach. *N*-Prop-2-enals, which are absorbed from the gut, are also formed in neutral or acidic aqueous media, but most of the absorbed material is not metabolised. A third type of reaction products are unavailable 4-substituted 1,4-dihydropyridine-3,5-dicarbaldehydes, which arise in reactions of malondialdehyde with amino compounds, such as lysine, in the presence of alkanals. Examples of malondialdehyde reaction products with lysine are N^{ε}-(prop-2-enal)lysine, so-called MDA-lysine, N^{α}-(prop-2-enal)lysine, *N*,*N*′-di(prop-2-enal)lysine (**3-150**) and conjugated cross-link in proteins termed lysine-MDA-lysine. An example of the reaction product of lysine with malondialdehyde and acetaldehyde is *N*,*N*′-di(4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde)lysine, which, for example, arises in the reaction of bovine serum albumin with malondialdehyde and acetaldehyde (**3-151**).

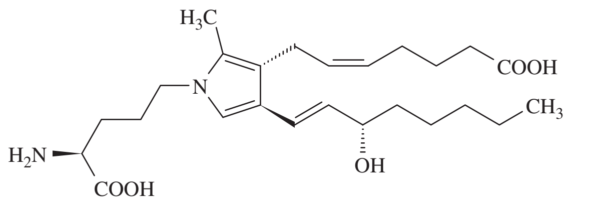


3-151, *N*,*N*′-di(4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde)lysine

ALEs can also be formed from prostaglandin H_2 (PGH_2), which rearranges non-enzymatically into levuglandin E_2. Levuglandin E_2 condenses with the ε-amino group of protein-bound lysine to form a pyrrole derivative levuglandin E_2-lysine (**3-152**), which has been identified in the blood plasma.



3-150, malondialdehyde conjugates with lysine



3-152, levuglandin E_2-lysine

Alk-2-enals also react to form adducts with 2′-deoxyguanosine (the corresponding base guanine) and other bases in DNA. If the damaged DNA is not repaired, these adducts may be mutagenic. The proposed reaction mechanism involves a Michael addition of the N^2-amino group of deoxyguanosine at the C-3 position of the alk-2-enals. The Michael product then cyclises at the *N*-1 of deoxyguanosine to form two pairs of diastereomers. The simplest alk-2-enal, acrolein, reacts with 2′-deoxyguanosine in DNA to form 1,N^2-propanodeoxyguanosine adducts: (6*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-6-hydroxypyrimido[1,2-*a*]purine-10(3H)/ones (Figure 3.75) and also (8*R*/*S*)-3-(2′-deoxyribos-1′-yl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10(3H)/ones. Other alk-2-enals, such as (*E*)-4-hydroxyhex-2-enal and (*E*)-4-hydroxynon-2-enal, react with deoxyguanosine analogously. Malondialdehyde possibly reacts with deoxyguanosine in a different way (via adenine adduct) to form a variety of adducts, the most abundant of which is pyrimido[1,2-*a*]purin-10(3*H*)-one, which is known as M_1G (Figure 3.75). The M_1G adduct is mutagenic and has been detected in human liver cells and other products.

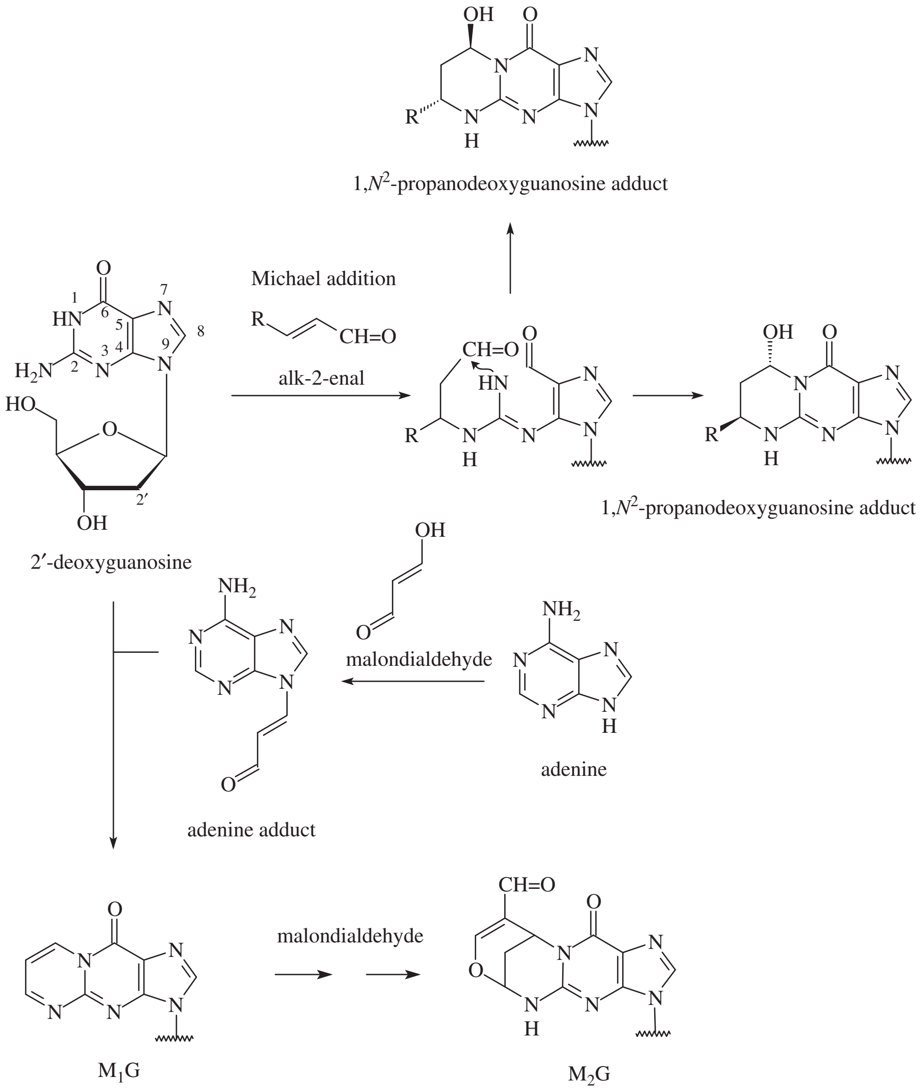


Figure 3.75 Formation of 2′-deoxyguanine adducts with alk-2-enals and malondialdehyde: R = H (acrolein), R = $CH(OH)CH_2CH_3$ (4-hydroxyhex-2-enal, R = $CH(OH)[CH_2]_4CH_3$ (4-hydroxynon-2-enal).

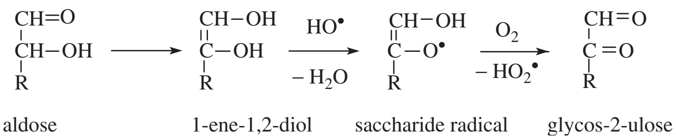


Figure 3.76 Oxidation of monosaccharides to glycosuloses.

3.8.1.12.2 Reactions with saccharides

Oxidation of saccharides is usually initiated by one-electron oxidising agents, such as transition metal ions (especially Fe^{3+} and Cu^{2+}) and free radicals (mainly the hydroxyl radical $HO^{\bullet}$ and alkoxyl radical $RO^{\bullet}$). The primary products of oxidation by free radicals (Figure 3.76), which takes place after the isomerisation to 1-ene-1,2-diols, are α-dicarbonyl compounds containing the original number of carbon atoms, known as glycos-2-uloses. Glycos-2-uloses are further oxidised and decomposed. Radicals formed as intermediates and α-dicarbonyl compounds react further with proteins in the Maillard reaction.

3.8.1.12.3 Reactions with vitamins

Vitamins that have a role as antioxidants (vitamin E, ascorbic acid and provitamins A) are easily oxidisable substances that react with free radicals or act as singlet oxygen scavengers. The reaction of tocopherols (T–OH) with hydroperoxyl radicals ($ROO^{\bullet}$) is also a part of the protective mechanisms in biomembranes of living tissues:

$$R\text{–}O\text{–}O^{\bullet} + T\text{–}OH \rightarrow R\text{–}O\text{–}OH + T\text{–}O^{\bullet}$$
$$(R\text{–}O\text{–}OH = \text{hydroperoxide}, T\text{–}O^{\bullet} = \text{tocopheryl radical})$$

At the phase interface, tocopheryl radicals are then reduced back to tocopherols by ascorbic acid (H_2A), which is oxidised to ascorbyl radical ($HA^{\bullet}$):

$$H_2A + T\text{–}O^{\bullet} \rightarrow HA^{\bullet} + T\text{–}OH$$

Another one-electron oxidation of ascorbyl radical produces dehydroascorbic acid (A), which is reduced back to ascorbic acid by dehydroascorbate reductase:

$$2HA^{\bullet} + NADH + H^{+} \rightarrow 2H_2A + NAD^{+}$$

Other vitamins are also oxidised. In folacin, for example, the links C-9 and N-10 are cleaved (see Section 5.12.2); vitamins D undergo photodegradation in the presence of photosensitisers (see Section 5.3.6).

3.8.1.12.4 Reactions with other compounds

Other significant reactions are reactions of oxidised lipids with phenolic compounds, many of which are used as antioxidants (see Section 11.2.2).

3.8.1.13 Oxidation in biological systems

Triplet oxygen is reduced to water during oxygenic photosynthesis in plants and in analogous reactions in the respiratory chain of animals[25]:

$$^3O_2 + 4\,e^- + 4H^+ \rightarrow 2\,H_2O$$

At the same time, toxic forms of oxygen, free radicals and covalent compounds, form as byproducts (through one, two and three-electron reduction), which oxidise lipids of biological membranes, then subsequently DNA, proteins and other biomolecules (Figure 3.77). Fortunately, organisms are also equipped with detoxifying mechanisms, which include:

- enzymes whose cofactors are Mn, Zn, Cu, Se and Fe
- vitamins (vitamin E, provitamins A and vitamin C).

3.8.1.13.1 Singlet oxygen

Singlet oxygen, which originates in photosensitised reactions (Figure 3.59), is very effectively scavenged by carotenes, tocopherols (vitamin E) and ascorbic acid (vitamin C) in living organisms as well as in edible oils and fats.

3.8.1.13.2 Superoxide anion

Superoxide anion O_2^- has one unpaired electron; therefore it is a free radical, $O_2^{-\bullet}$. This superoxide radical arises by one-electron reduction of triplet oxygen. In plants, this reaction is catalysed by

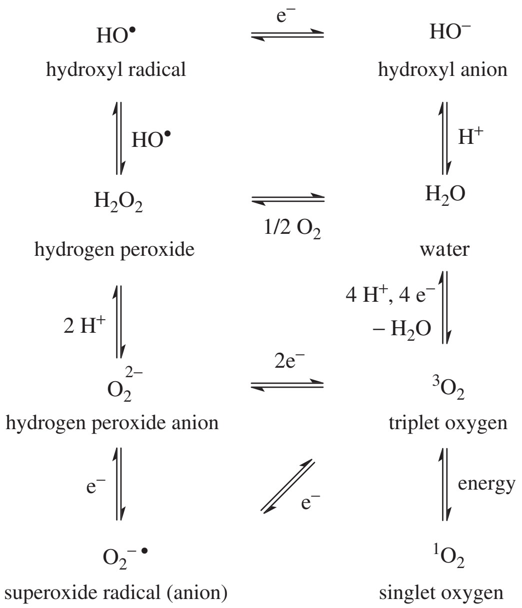


Figure 3.77 Metabolism of oxygen.

[25]During oxygenic photosynthesis, the reaction $A + H_2O + \text{energy} \rightarrow AH_2 + \frac{1}{2}O_2$ takes place, where A is the acceptor (carbon dioxide) and AH_2 the monose unit ($H_2C{=}O$). The oxidation of nutrients in the respiratory chain proceeds according to the equation: $AH_2 + \frac{1}{2}O_2 \rightarrow A + H_2O + \text{energy}$ (e.g. the oxidation of sugars in the citric acid cycle and oxidation of fatty acids by β-oxidation).

NADPH oxidase, in animals superoxide radical is a byproduct of other oxidases):

$$^3O_2 + e^- \rightarrow O_2^{-\bullet}$$

The superoxide anion is removed by the activity of superoxide dismutase. The mitochondrial enzyme contains Mn as a cofactor; the cytosolic enzyme has Zn and Cu as cofactors. A donor of two hydrogen atoms may be, for example, ascorbic acid ($H_2A + O_2^{-\bullet} + H^+ \rightarrow HA^\bullet + H_2O_2$):

$$O_2^{-\bullet} + e^- \rightarrow O_2^{2-}$$
$$O_2^{2-} + 2H^+ \rightarrow H_2O_2$$

Spontaneously, without the involvement of any enzymes, one-electron reduction or dismutation (the so-called Haber–Weiss reaction) takes place:

$$O_2^{-\bullet} + H_2O_2 \rightarrow HO^\bullet + HO^- + {}^3O_2$$
$$O_2^{-\bullet} + Fe^{3+} \rightarrow {}^3O_2 + Fe^{2+}$$

3.8.1.13.3 Hydrogen peroxide

Hydrogen peroxide is the result of two-electron reduction of triplet oxygen:

$$^3O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

It is mainly decomposed by microsomal catalase ($H_2O_2 : H_2O_2$ oxidoreductase), which contains Fe (haematin) as a cofactor or by cytoplasmic or mitochondrial GSH peroxidase (H_2O_2 : donor oxidoreductase) containing Se as a cofactor:

$$2H_2O_2 \rightarrow 2H_2O + {}^3O_2$$
$$2G\text{–}SH + H_2O_2 \rightarrow G\text{–}S\text{–}S\text{–}G + 2H_2O$$

Otherwise, a single electron reduction takes place, which proceeds as in the Haber–Weiss reaction or Fenton reaction, which yields a highly reactive hydroxyl radical ($HO^\bullet$):

$$H_2O_2 + e^- \rightarrow HO^\bullet + HO^-$$
$$H_2O_2 + Fe^{2+} \rightarrow HO^\bullet + HO^- + Fe^{3+}$$

3.8.1.13.4 Hydroxyl radical

The most dangerous toxic form of oxygen is the hydroxyl radical, which is generated by two-electron reduction of triplet oxygen:

$$^3O_2 + 3e^- + 3H^+ \rightarrow HO^\bullet + H_2O$$

Hydroxyl radicals damage the mitochondrial (chromoplastic), microsomal (peroxysomal) and endoplasmic reticulum membranes. The damage associated with many degenerative processes (diseases) includes certain forms of cancer and occurs even during the normal aging of organisms. Hydroxyl radicals operate mainly at the place of formation, where they attack unsaturated fatty acid (RH) bound in the membrane phospholipids. Unsaturated fatty acids yield hydroperoxides (R–O–OH) through the previously described mechanisms:

$$R\text{–}H + HO^\bullet \rightarrow R^\bullet + H_2O$$
$$R^\bullet + O_2 \rightarrow R\text{–}O\text{–}O^\bullet$$
$$R\text{–}O\text{–}O^\bullet + R\text{–}H \rightarrow R\text{–}O\text{–}OH + R^\bullet$$

The protective mechanism of organisms lies in the reduction of hydroperoxyl radicals by vitamin E (tocopherols, TH):

$$R\text{–}O\text{–}O^\bullet + T\text{–}H \rightarrow R\text{–}O\text{–}OH + T^\bullet$$

Tocopheryl radicals $T^\bullet$ are stabilised by a number of reactions. They can be reduced back to tocopherols by ascorbic acid (H_2A), which is oxidised to dehydroascorbic acid (A):

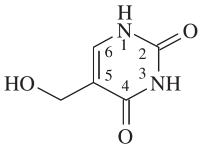
$$2T^\bullet + H_2A \rightarrow 2T\text{–}H + A$$

Hydroperoxides are removed from bonds in damaged phospholipid by activated phospholipase A_2 and free hydroperoxide becomes a substrate for peroxidases. Otherwise, the oxidation of fatty acids results in the accumulation of lipid hydroperoxides and their secondary metabolites such as (*E*)-4-hydroxynon-2-enal and malondialdehyde, which react with proteins and DNA (see Section 3.8.1.12.1).

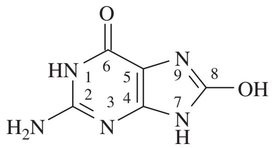
Proteins and deoxyribonucleic acids

The main agents damaging proteins are hydroxyl radicals ($HO^\bullet$), singlet oxygen ($^1O_2$) and superoxide radicals ($HO_2^\bullet$). Reactions are analogous to those in foods and can result in loss of enzymatic activity, cell cytolysis and cell death. Damaged proteins in biomembranes are often associated with oxidised lipids.

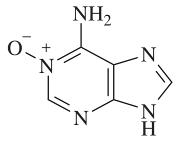
The main oxidising agent of DNA is the hydroxyl radical ($HO^\bullet$), which oxidises the purine and pyrimidine bases and deoxyribosyl residues. The main oxidation products are 5-(hydroxymethyl)uracil (**3-153**) and 8-hydroxyguanine (**3-154**), which is formed by reaction of guanine with singlet oxygen. In the presence of transition metals, the main compound that is attacked is adenine. The reaction product is adenine-N^1-oxide (**3-155**).



3-153, 5-(hydroxymethyl)uracil



3-154, 8-hydroxyguanine



3-155, adenine-N^1-oxide

3.8.1.14 Degradation reactions

At high temperatures, pyrolytic reactions of lipids can yield fatty acid anhydrides, and fatty acids may eliminate carbon dioxide by decarboxylation. These reactions take place during food processing only at trace levels.

3.8.2 Reactions of homolipids

3.8.2.1 Reactions of waxes

Waxes do not have specific chemical properties and their reactivities are similar to other fatty acid esters. The only difference is that, due to their higher molecular weight and high melting points, waxes react more slowly. Their most important chemical reaction is hydrolysis to the parent components, primary alcohols and fatty acids.

3.8.2.2 Reactions of fats and oils

3.8.2.2.1 Hydrolysis and saponification

Hydrolysis of fats and oils (lipolysis) in foods is primarily caused by the action of lipases. The sources of lipases can be crude oils (lipase from vegetable seeds, beans or fruit pulps) or microorganisms. Some lipases show substrate specificity according to the length of the acyl group and regiospecificity (*sn*-non-specific and *sn*-1,3-specific lipases). Lipolysis in food technology may proceeds on purpose, for example, during ripening of certain cheeses, with specific types of molds added during the cheesemaking process (surface-ripened Camembert or blue chesses such as Roquefort and Gorgonzola with molds growing in the curd), and in cured and fermented dried sausages (by *Lactobacillus* bacteria and other bacteria added as starter cultures or by natural microflora). Examples are given in Table 3.47.

Hydrolysis products are dependent on the specificity of lipase. In the case of pancreatic lipase, the final products are fatty acids and 2-monoacylglycerols, in the case of *sn*-non-specific lipases from oil seeds, di- and monoacylglycerols, glycerol and free fatty acids gradually form upon hydrolysis of crude vegetable oils. A necessary condition for the enzymatic hydrolysis process is the presence of water. The solubility of water in refined oils is around 0.1% at 20 °C, and may be higher in crude oils due to the presence of heterolipids. These water contents are sufficient for hydrolysis. Analogously, hydrolysis takes place in the fat cells of oilseeds with increased water content. Enzymatic hydrolysis is used, for example, to obtain essential fatty acids containing C_{20}–C_{22} fatty acids, when high temperatures cannot be used.

Partial hydrolysis of fats actually occurs at high temperatures during deep frying of foods, when the released water causes hydrolysis of the ester bonds. The released fatty acids distil off from the oil bath to the surroundings. Particularly prone to this behaviour are fats containing medium-chain fatty acids, such as coconut or palm kernel oil and milk fat.

Fatty acids in oleochemistry (as well as fatty acids for food use) are obtained through autocatalysed hydrolysis (Figure 3.78) at high temperatures, where the byproduct is glycerol. Temperatures above 200 °C are particularly necessary in order to increase the solubility of water in the fat. The solubility of water in fat generally depends on temperature and the length of the acyl group. For example, water solubility at 93 and 205 °C in coconut oil is 3.5 and 12%, while in beef tallow it is only 1 and 5%, respectively. The reaction proceeds in a two-phase system of oil–water, where the lipid phase is a dispersion medium. Glycerol is soluble in water and is extracted from the fat phase into the aqueous phase; therefore the reaction equilibrium is shifted in favour of hydrolysis. Using a continuous counter flow arrangement, a degree of hydrolysis of up to 98–99% can be achieved.

Saponification of lipid ester bonds is based on the reaction of aqueous solutions of alkali metal hydroxides and the formation of soaps (alkali metal salts of fatty acids) and glycerol as a byproduct. Intermediates of saponification of triacylglycerols are di- and monoacylglycerols.

A partial saponification of acylglycerols also occurs during deacidification (neutralisation) of raw oils by alkaline agents, which

Table 3.47 Specific lipases from different sources.

Lipase source	Specificity to acyl length[a]	Regiospecificity (*sn*)	Lipase source	Specificity to acyl length[a]	Regiospecificity (*sn*)
Pancreatic lipase	S > M, L	1,3	*Penicillium camembertii*	MAG, DAG > TAG[b]	1,3
Stomach lipase	S, M ≫ L	1,3	*Penicillium roqueforti*	S, M ≫ L	1,3
Aspergillus niger	S, M, L	1,3 ≫ 2	*Rhizopus javanicus*	M,L > S	1,3 > 2
Candida lipolytica	S, M, L	1,3 > 2	*Rhizopus japonicus*	S, M, L	1,3 > 2
Candida cylindracea	S, M, L	1,2,3	*Rhizopus oryzae*	M,L > S	1,3 ≫ >2
Rhizomucor mihei	S > M, L	1 > 3 ≫ 2	*Pseudomonas fluorescens*	M,L > S	1,3 > 2
Mucor javanicus	M,L ≫ S	1,3 > 2	*Pseudomonas* spp.	S, M, L	1,3 > 2

[a] L = long chain fatty acids, M = medium chain fatty acids, S = short chain fatty acids, MAG = monoacylglycerols, DAG = diacylglycerols, TAG = triacylglycerols.
[b] Substrate specificity to acylglycerols.

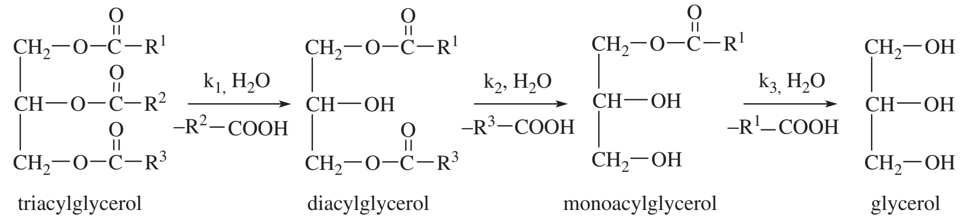


Figure 3.78 Hydrolysis (saponification) of fats (reaction rate constants $k_3 > k_2 > k_1$).

is a side-reaction that leads to some loss of oil. A partial saponification also takes place during ester exchange, when sodium hydroxide is used as a catalyst. Saponification of fat blends is used deliberately to produce sodium soaps, toilet soaps (from a mixture of coconut oil and beef tallow) and detergent soap powders (usually from beef tallow only).

3.8.2.2.2 Interesterification reactions

In oleochemistry, a characteristic reaction of fatty acids is esterification, but the characteristic reaction of their esters, especially natural fats, is an interesterification reaction. Interesterification is a set of reactions where the original ester reacts with a fatty acid, an alcohol or another ester.[26] The following reactions are seen:

- **acidolysis**
- **alcoholysis**
- **ester interchange (transesterification)**.

This set of reactions is catalysed either by enzymes or acidobasic catalysts and is characterised by migration of acyls between the molecules of alcohols or in the glycerol molecule. When using acid–base catalysts, acyl migration is statistically random. There is a growing interest in using enzymes (lipases or phospholipases) in technological applications, where it is possible to perform selective interesterification reactions catalysed by specific enzymes.

In the presence of water, lipases catalyse the hydrolysis of lipids to fatty acid partial esters and, in the final stage, to glycerol. Hydrolysis is the opposite reaction of the esterification reaction catalysed by lipases, where the resulting water has to be removed.[27] Esterification is used industrially for the preparation of fatty acid esters. In the absence of water, lipases (as well as other hydrolases) also catalyse the interesterification reaction. When immobilised lipases became available commercially, this reaction found applications on an industrial scale. The basic advantage of the use of lipases, compared with acidobasic catalysts, is their specificity, particularly if particular *sn*-1,3-lipases are employed.

Acidolysis

Acidolysis is a reaction of triacylglycerols with carboxylic acid, which leads, under acid catalysis, to the exchange of acyl residues. The reaction is in dynamic equilibrium and if the equilibrium is not shifted by removing reaction components, the product contains all components, including the starting reactants (Figure 3.79). Of all the interesterification reactions, acidolysis currently has the least practical significance.

The reaction of C_{16} and C_{18} higher fatty acids with triacylglycerols of coconut oil results in the release of C_8–C_{14} fatty acids, which can be continuously distilled from the reaction mixture as the product. The reaction can be autocatalysed at higher temperatures or an acidic catalyst (such as 4-toluenesulfonic acid) as well as basic catalysts (such as zinc, calcium or magnesium oxides) may be used. These procedures are now obsolete and fatty acids (for food purposes, for the production of triacylglycerols containing caprylic and capric acids only) are now obtained by hydrolysis and subsequent rectification.

[26]Sometimes, the term transesterification is used instead of alcoholysis, particularly instead of methanolysis. It is also possible to come across the term re-esterification, which is also used in several senses. Oleochemistry uses this term for interesterification and esterification of fatty acids with glycerol.

[27]Water activity must be about 0.3, when no changes in the tertiary structure of the enzyme causing loss of activity occur. At this value, complexes of enzyme–substrate and enzyme–fatty acid may be formed and the latter complexes are cleaved by water.

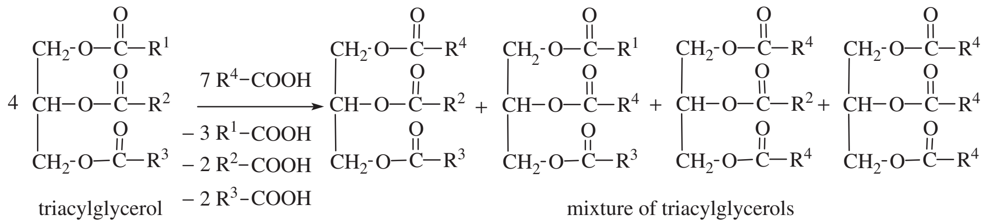
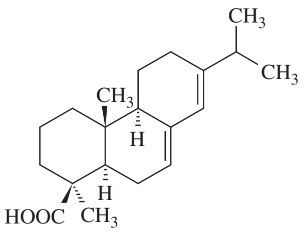


Figure 3.79 Acidolysis of triacylglycerols.

Acidolysis of fatty acids with diterpene abietic acid (**3-156**), the main component of the so-called resin acids (see Section 8.2.14) is used, for example, to produce varnishes, where the exchange of fatty acids for phthalic acid yields glyptals (polymers containing the ester functional group with properties of natural resins).



3-156, abietic acid

An important application of enzymatic acidolysis, specifically using *sn*-1,3-lipase, is the synthesis of structured triacylglycerols containing palmitic or stearic acid in the *sn*-1 and *sn*-3 positions and oleic acid in the *sn*-2 position. These triacylglycerols are components of cocoa butter (Figure 3.80). The synthesis is a consequence of a fraction of palm oil containing 1,2-dipalmitoyl-2-oleoyl-*sn*-glycerol. Acidolysis with stearic acid gives a mixture of 1-palmitoyl-2-oleyl-3-stearoyl-*sn*-glycerol and 1,3-distearoyl-2-oleoyl-*sn*-glycerol. The third major triacylglycerol contained in cocoa butter is obtained by acidolysis of trioleoylglycerol (triolein) with stearic acid. Combination of both procedures gives a mixture of structured triacylglycerols, the so-called CBE fat (cocoa butter equivalent), which serves as a substitute for cocoa butter.

Enzymatically catalysed acidolysis using specific *sn*-1,3-lipases is used for the preparation of dietetically important structured triacylglycerols of the MUM-type (Figure 3.81) that contain unsaturated (U) essential fatty acids in the outer *sn*-1,3 positions and medium-chain (M) fatty acids (caprylic or capric) in the *sn*-2 position.

$$\text{POP} + \text{St} \xrightleftharpoons{sn\text{-1,3-specific lipase}} \text{POSt} + \text{StOSt} + \text{P}$$

$$\text{OOO} + \text{St} \xrightleftharpoons{sn\text{-1,3-specific lipase}} \text{StOSt} + \text{O}$$

Figure 3.80 Enzymatic acidolysis of CBE triacylglycerols: POP = 1,3-dipalmitoyl-2-oleoyl-*sn*-glycerol, POSt = 1-palmitoyl-2-oleoyl-3-stearoyl-*sn*-glycerol, StOSt = 1,3-distearoyl-2-oleoyl-*sn*-glycerol, P = palmitic acid, St = stearic acid, O = oleic acid.

$$\text{UUU} + \text{M} \xrightleftharpoons{sn\text{-1,3-specific lipase}} \text{MUM} + \text{U}$$

Figure 3.81 Enzymatic acidolysis of unsaturated triacylglycerols: U = triacylglycerol containing unsaturated fatty acids (oleic acid or essential acids), M = caprylic or capric acid.

Alcoholysis

The reaction of triacylglycerols with alcohol is called alcoholysis. It can be catalysed by acids (sulfuric acid or 4-toluenesulfonic acid), and then an interesterification reaction, which is a parallel reaction to esterification of free fatty acids occurring in the fat. Alcoholysis is kinetically similar to esterification. The base catalysed reaction employs methoxides, hydroxides and carbonates of alkali metals. The most commonly used catalysts are sodium methoxide, sodium hydroxide or potassium hydroxide. Industrially produced alcoholysis products are methyl, ethyl or propyl esters (Figure 3.82) of fatty acids. Analogous to hydrolysis, mixing of fat (oil) with alcohol gives a two-phase system

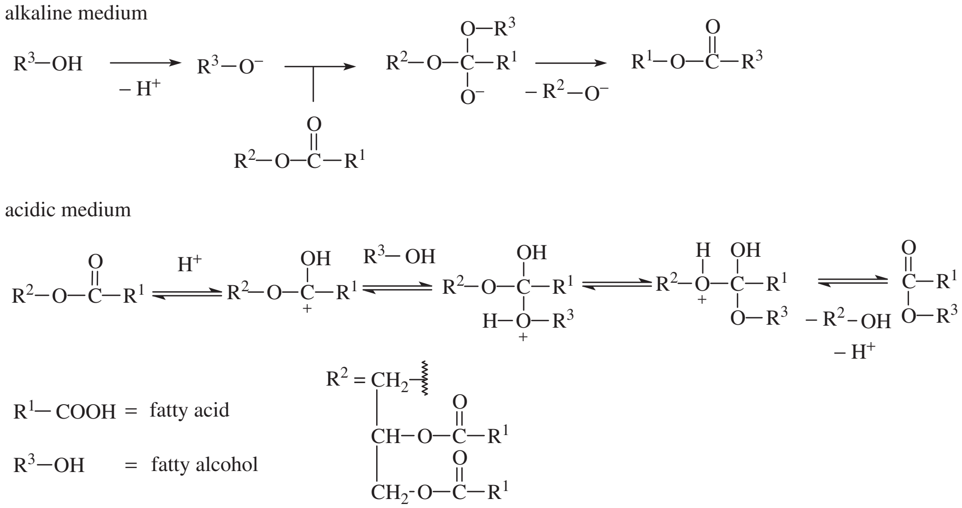


Figure 3.82 Mechanism of alcoholysis of triacylglycerols.

consisting of the alcohol (glycerol) phase and ester phase. The ester phase is a homogeneous reaction phase in which glycerol dissolves, to a limited extent, and passes into the alcohol phase. It is therefore possible to achieve a significant shift in the reaction equilibrium; the degree of conversion is 98–99%.

Vegetable oils (such as rapeseed, soybean and palm oils) or animal fats (beef tallow) are used for the production of so-called biodiesel, which is typically made by alcoholysis of oils and fats with methanol, ethanol or propan-1-ol. With the growth in the hydrocarbon chain length of the alcohol, the lipophilicity increases so that the two-phase system does not form in the reaction with propan-1-ol, and glycerol does not separate. Therefore, the industrially important esters, such as propyl or butyl esters as well as esters of secondary alcohols, can be only prepared by direct esterification of fatty acids with the appropriate alcohol. Methyl and especially ethyl esters can also be obtained by enzyme-catalysed alcoholysis using non-specific lipases.

Glycerolysis

Glycerolysis is a particular example of alcoholysis, being a reaction in which fat (triacylglycerols) reacts with glycerol (Figure 3.83) to give a mixture of monoacylglycerols and diacylglycerols. Glycerolysis takes place at high temperatures (at temperatures 200–250 °C the solubility of glycerol in fat increases to 220–400 moles per one mole of fat) in the presence of an alkaline catalyst (usually either potassium hydroxide, calcium oxide or sodium methoxide). The excess of glycerol is chosen to correspond to its solubility at a given temperature, for example 220–400 moles/mole of triacylglycerols; nevertheless the reaction mixture contains less than 55–60% of monoacylglycerols that are used as emulsifiers. This mixture can be used as such, but for more demanding applications the reaction mixture is subjected to molecular distillation, which gives the product (emulsifier) containing up to 95% of monoacylglycerols (Table 3.48). These emulsifiers also contain varying fatty acids compositions. The composition of the emulsifier is usually close to the fatty acid composition of beef tallow, even though they are usually made from plant oils. The process of glycerolysis has

Table 3.48 Composition of monoacylglycerol emulsifiers.

Component (%)	Emulsifier[a]	Distilled emulsifier[b]
Monoacylglycerols	50–55	90–95
Diacylglycerols	30–35	2–5
Triacylglycerols	<5	0.2–1.0
Free fatty acids	1.0	1.0–2.0
Glycerol	<8	0.2–1.0

[a] The emulsifier composition corresponds to the composition of the fat phase after glycerolysis.
[b] Molecularly distilled emulsifier.

industrial importance, because emulsifiers produced in this way are used in the food, cosmetics and pharmaceutical industries. Emulsifiers based on monoacylglycerols can also be produced by direct esterification of fatty acids with glycerol, but this method is only rarely used.

Glycerolysis can also be catalysed by specific *sn*-1,3-lipases (Figure 3.84). A mixture of di- and monoacylglycerols is obtained in the first stage; further glycerolysis gives products with a reduced diacylglycerols content, which in practice is a mixture of 1- and 2-monoacylglycerols. Isolated 2-monoacylglycerols can also be used for the synthesis of structured triacylglycerols.

A special case of alcoholysis is the use of sugars, usually saccharose and sugar alcohols. Direct esterification of sucrose is very difficult, because it leads to dehydration and caramelisation (see Section 4.7.6). Alcoholysis may, depending on the initial fatty acid ester, give different products. The reaction of saccharose with a mixture of triacylglycerols gives a mixture of saccharose esters with mono-, di-, triacylglycerols and glycerol. As saccharose has a total of eight hydroxyl groups, compounds ranging from saccharose mono to octa fatty acid esters can be produced. This reaction mixture is known by the name **sugar esters**. Sugar esters are mixed non-ionic surfactants (see Section 11.5.2.1.5) widely used as emulsifiers in

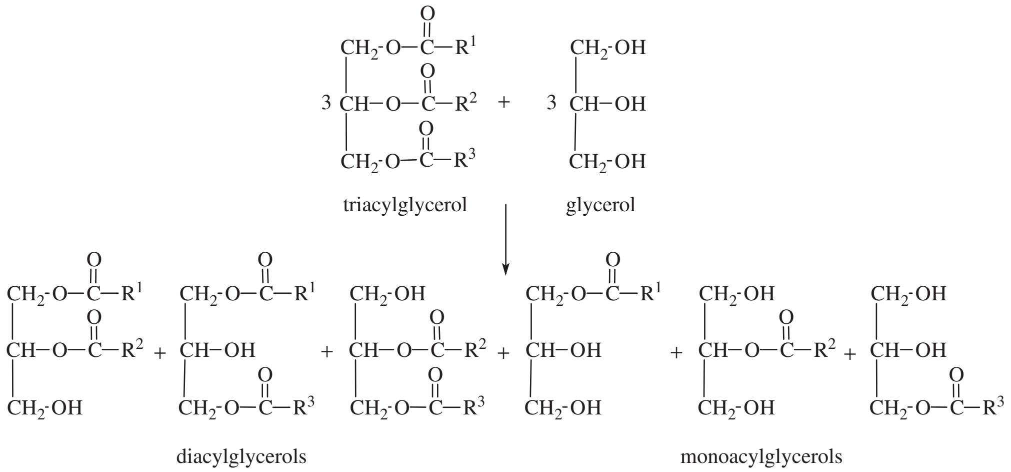


Figure 3.83 Glycerolysis of triacylglycerols.

$$\mathrm{TAG + G} \xrightleftharpoons{sn\text{-}1,3\text{-specific lipase}} \mathrm{1,2\text{-}DAG + 2,3\text{-}DAG + 2\text{-}MAG} \xrightleftharpoons{sn\text{-}1,3\text{-specific lipase}} \mathrm{1(3)\text{-}MAG + 2\text{-}MAG}$$

Figure 3.84 Glycerolysis of triacylglycerols by specific lipases.

foods and beverages, in detergents, in industrial cleaners, in agricultural chemicals and as excipients in pharmaceuticals. Products consisting of sucrose monoesters, and to a small extent sucrose diesters, are manufactured by a transesterification reaction of sucrose and fatty acid methyl esters in *N*,*N*-dimethylformamide or dimethylsulfoxide.

The maximum degree of esterification gives a mixture in which hexa, hepta and octa sucrose esters prevail. The product Olestra is a lipid-like substance that was developed as a non-energy (non-absorbable) fat substitute, since ester bonds are not hydrolysed by enzymes of the digestive tract. However, it was found that Olestra has some adverse gastrointestinal effects and depletes blood levels of many valuable fat-soluble substances, including carotenoids.

Ester interchange

The ester interchange (or transesterification) is catalysed by alkaline catalysts. For food uses, sodium hydroxide or sodium methoxide are usually used. The choice of catalyst is closely related to reaction temperature. Sodium methoxide is effective in the temperature range of 50–70 °C and the maximum transesterification temperature is 120 °C, while sodium hydroxide is effective above 160 °C. The maximum temperature for the catalyst must be chosen so that in the aprotic environment the condensation reaction of esters is avoided. The reaction is rapid and in the homogeneous phase proceeds to equilibrium according to the diagram in Figure 3.85. The equilibrium composition of the reaction mixture that is achieved will not be affected by changes in composition of the reaction mixture components, as a new equilibrium is always reached. This is why this transesterification is called uncontrolled transesterification. In the resulting equilibrium mixture, the distribution of fatty acids in triacylglycerols is random, very different from the fatty acid distribution in natural triacylglycerols, where the genetic information is generally encoded in DNA. Therefore, this transesterification is often also known as **randomisation**.

The composition of the randomised reaction mixture of triacylglycerols can be calculated by the number of triacylglycerols present. The number of triacylglycerols (taking into consideration positional isomers) is equal to $(n^3 + n^2)/2$ and the number of triacylglycerols without positional isomers is $(n^3 + 3n^2 + 2n)/6$. When 5 (6) acyls are present in fats, which is typical in triacylglycerol mixtures, the total number of triacylglycerols after randomisation is 75 (126) and 35 (56), respectively.

The only way to affect the shift in the reaction equilibrium during randomisation is the so-called controlled transesterification, where the reaction temperature is chosen so that triacylglycerols with the highest melting points crystallise, which changes the original composition of the reaction mixture of triacylglycerols and a new equilibrium is created in the liquid phase. This procedure, which can be repeated, is a combination of randomisation with fractional crystallisation of triacylglycerols. At temperatures of crystallisation of triglycerides, at which sodium methoxide is not effective, a eutectic alloy of sodium with potassium has to be used. The actual catalysts are then sodium and potassium mono- and diacylglycerolate anions that form in the reaction of alkali metals with the mono- and diacylglycerols that are present in small amounts in natural fats.

In base-catalysed transesterification, some side reactions take place. For example, the reaction of water with sodium methoxide creates sodium hydroxide that can cleave the ester bonds to form soaps. Oxidation of tocopherols and other antioxidants in alkaline media also occurs, which reduces the oxidative stability of transesterified fat.

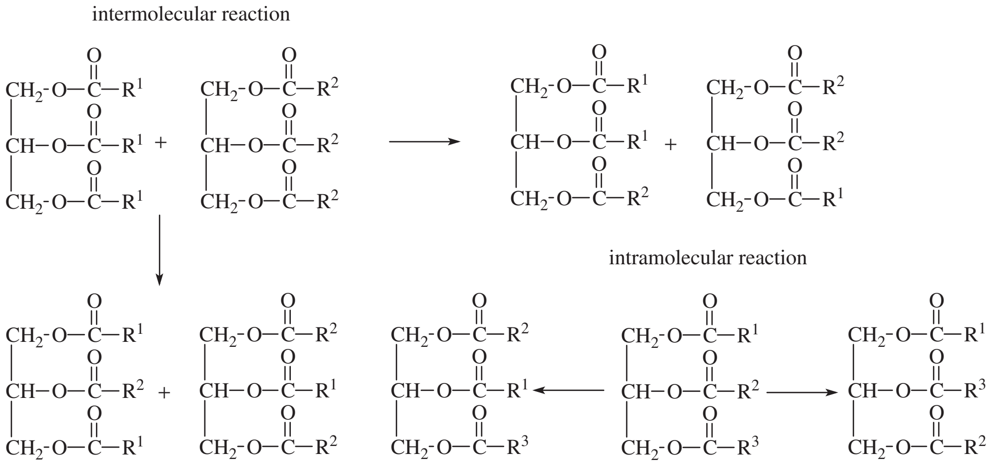


Figure 3.85 Ester interchange in triacylglycerols.

As it is now undesirable to produce fats (fat blends) containing *trans*-isomers of C_{18} fatty acids, which implies the complete elimination of the partial catalytic hydrogenation technology of oils and fats, uncontrolled transesterification has become the only method of modification of triacylglycerols leading to the production of fats of suitable consistency. **Structural fat** is in this case prepared by an alkali-catalysed uncontrolled transesterification of at least two different fats. One possibility is the preparation of structural fat based on fully hydrogenated vegetable fats, the first of which is the source of medium chain fatty acids (coconut or palm kernel oil), and the second is the source of long chain fatty acids (such as fully hydrogenated rapeseed oil). The ratio of the two fats is chosen so that the content of triacylglycerols containing saturated fatty acids has no adverse effect on the texture of the resulting fat blend. This means that the acyls of saturated fatty acids substitute acyls of *trans*-C_{18} fatty acids in the original partially hydrogenated vegetable oils. The total content of *trans*-isomers in fat blends based on transesterified fats does not usually exceed 1% of the total fatty acid content.

Enzymatically catalysed transesterification

The mechanism of acyl exchange during enzymatic transesterification of triacylglycerols is explained by the formation of the complexes enzyme–enzyme–acylglycerol and enzyme–fatty acid (Figure 3.86). A substantial change in the use of lipases acting as transesterification catalysts is their substrate (group) specificity (depending on the acyl length) and especially the regiospecificity of *sn*-1,3-specific lipases. As a result of their *sn*-1,3-regiospecifity, the randomisation of acyls occurs only in the *sn*-1 and *sn*-2 positions of triacylglycerol molecules (Figure 3.87) and a substantial portion of unsaturated fatty acids remains in the *sn*-2 position, which is considered from a nutritional point of view as an asset. Structural fats derived from enzyme catalysis have different physical properties, including texture, unlike the structural fats obtained by base-catalysed randomisation.

Enzymatic transesterification of triacylglycerols does not lead to the oxidation of tocopherols. The actual catalyst is sensitive to the presence of hydroperoxides and aldehydes, which may cause changes in the enzyme structure. This also leads to a gradual loss of its activity.

3.8.2.2.3 Pyrolytic reactions

Heating fat to high temperatures causes its pyrolysis in parallel with oxidation, polymerisation, oxypolymerisation and some other reactions. The presence of water leads to hydrolysis of fatty acids bound in triacylglycerols, and diacylglycerols, monoacylglycerols, glycerol and free fatty acids are formed as products. Free fatty acids and other products are also formed during pyrolysis of triacylglycerols in the absence of water (Figure 3.88). Another important product of pyrolysis is acrolein, that is produced in anhydrous fats, for example during deep frying, and which irritates eyes and mucous membranes of operating personnel. Acrolein is also produced directly by dehydration of glycerol.

$$TAG^1 + E \rightleftarrows TAG^1 \cdot E \rightleftarrows DAG^1 + FFA^1 \cdot E$$

$$TAG^2 + E \rightleftarrows TAG^2 \cdot E \rightleftarrows DAG^2 + FFA^2 \cdot E$$

$$DAG^1 + FFA^2 \cdot E \rightleftarrows TAG^3 \cdot E \rightleftarrows TAG^3 + E$$

$$DAG^2 + FFA^1 \cdot E \rightleftarrows TAG^4 \cdot E \rightleftarrows TAG^4 + E$$

$$TAG^3 + E \rightleftarrows$$

$$TAG^4 + E \rightleftarrows$$

$$FFA^1 \cdot E + H_2O \rightleftarrows FFA^1 + E$$

$$FFA^2 \cdot E + H_2O \rightleftarrows FFA^2 + E$$

Figure 3.86 Mechanism of enzymatically catalysed transesterification of triacylglycerols: TAG = triacylglycerol, DAG = diacylglycerol, MAG = monoacylglycerol, FFA = free fatty acid, E = enzyme.

$$AXA + BYB \xrightleftharpoons{sn\text{-}1,3\text{-specific lipase}} AXB + BXA + AYB + BYA$$

Figure 3.87 Transesterification of triacylglycerols catalysed by *sn*-1,3 specific lipase: A and B = acyls bound at *sn*-1,3-positions, X and Y = acyls bound at *sn*-2 positions.

3.8.3 Reactions of heterolipids

Reactions of heterolipids are generally similar to those of other lipids, but reactions of bound phosphoric acid are specific to heterolipids. Phospholipids are hydrolysed by various phospholipases; an overview of which is given in Table 3.47 and the mechanism of their action is shown in Figure 3.89. Phosphatidylcholine is a demanded product and its concentration in lecithin can be increased by enzymatically controlled interesterification after adding choline. Phosphatidylcholine can also be produced directly from diacylglycerols or phosphatidic acids using immobilised enzymes.

Phospholipids can form salts, for example with metal ions, due to the presence of one free hydroxyl group of the bound phosphoric acid in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol and two free hydroxyl groups in phosphatidic acid. Mostly, calcium and magnesium ions are involved in these reactions, but the resulting complexes have hydrophobic character, as in metal soaps. Cations of heavy metals (copper, manganese and iron) bound in phospholipids catalyse autoxidation significantly less than free metal ions, phospholipids can thus become synergists of antioxidants.

Unsaturated fatty acids bound in phospholipids can be oxidised by oxygen, similarly to fatty acids in triacylglycerols. Phospholipid hydroperoxides may react with amino groups of other phospholipids and other amino compounds to form dark brown macromolecular products similar to melanoidins (see Section 4.7.5.7) formed in the Maillard reaction. Therefore, phospholipids act as synergists (among other mechanisms) of

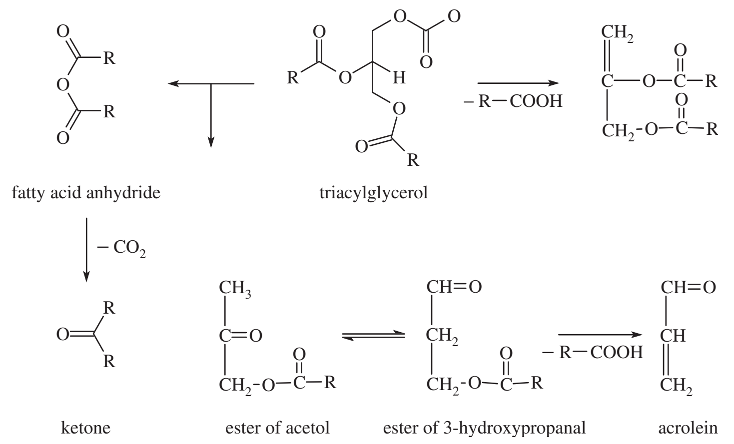


Figure 3.88 Formation of acrolein in triacylglycerol thermolysis.

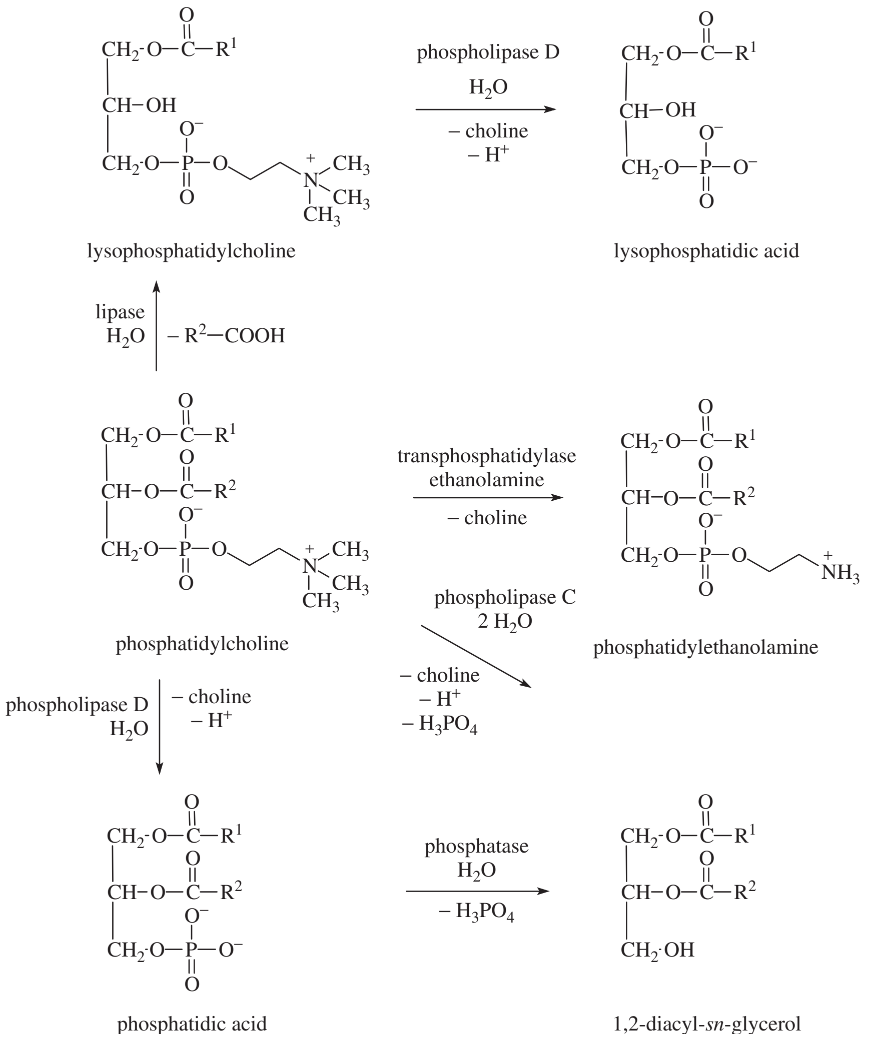


Figure 3.89 Degradation of phospholipids catalysed by phospholipases.

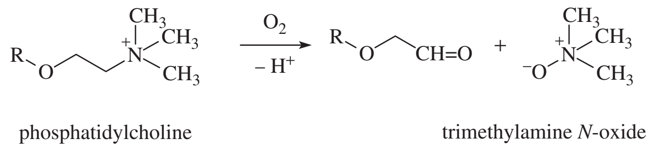


Figure 3.90 Oxidation of bound choline (R = residue of phospholipid molecule).

phenolic antioxidants. In addition to the chains of unsaturated fatty acids bound in phosphatidylcholine, the bound choline is also oxidised to trimethylamine *N*-oxide (Figure 3.90), which is further degraded to dimethylamine.

An important group of reactions of phospholipids containing free amino groups are nonenzymatic browning reactions of the amino groups with reducing sugars or with dehydroascorbic acid. The reaction mechanism is similar to the reaction of sugars with other amines. Phosphatidylethanolamine and phosphatidylserine are more reactive than phosphatidylcholine.

3.8.4 Reactions of steroids

Important reactions of free steroids include esterification, where esters and glycosides of steroid compounds are easily hydrolysed. These reactions in food raw materials are catalysed by sterol esterases and glycosidases, respectively. Other important reactions of steroids include elimination and substitution reactions and oxidation. Hydrogenation of steroids is of industrial importance.

3.8.4.1 Elimination and substitution reactions

When sterols are oxidised at moderate temperatures (≤100 °C), their reaction products are mainly derived from hydroperoxides, but at temperatures close to 200 °C and above, thermal reactions such as dehydration and condensation become important. The secondary hydroxyl groups are eliminated as water molecules, and new double bonds of Δ^5 sterols arise preferentially at the Δ^3 position, which results in the formation of sterol dienes with conjugated double bonds. For example, stigmasta-3,5-diene is produced by dehydration of β-sitosterol. The level of stigmasta-3,5-diene in virgin olive oils is very low (<0.1 mg/kg), but its concentrations in refined olive oils (pomace and olive oil mixtures) may reach ≤120 mg/kg. Refined oils also contain significant amounts of other steroidal hydrocarbons, including campesta-3,5-diene and stigmasta-3,5,22-triene in addition to stigmasta-3,5-diene. The relative amounts of these steroidal hydrocarbons can be used to detect refined seed oils.

The elimination of water may also proceed intermolecularly, which yields disteryl ethers (Figure 3.91). Disteryl ethers are formed in low concentration (a few milligrams per kilogram) by dehydration of sterols during the conventional bleaching of fats and oils with acid-activated bleaching earths. Disteryl ethers are the main steryl dimers characterised in foods; other dimers may be formed by direct sterol ring linkages.

In the production of acid hydrolysates of proteins using hydrochloric acid, the residual sterols and their esters occurring in the starting materials yield small amounts of 3-chloro derivatives by replacement of the C-3 hydroxyl group with chlorine. Possible intermediates in the formation of 3-chlorosterols may be 3,5-dienes derived from sterols (Figure 3.91).

3.8.4.2 Addition reactions

Double bonds of sterols can be hydrogenated similarly to double bonds of unsaturated fatty acids bound in triacylglycerols. Hydrogenation of oils and fats results in changes to the structure of steroids, especially in the molecules of 4,4-dimethylsterols and

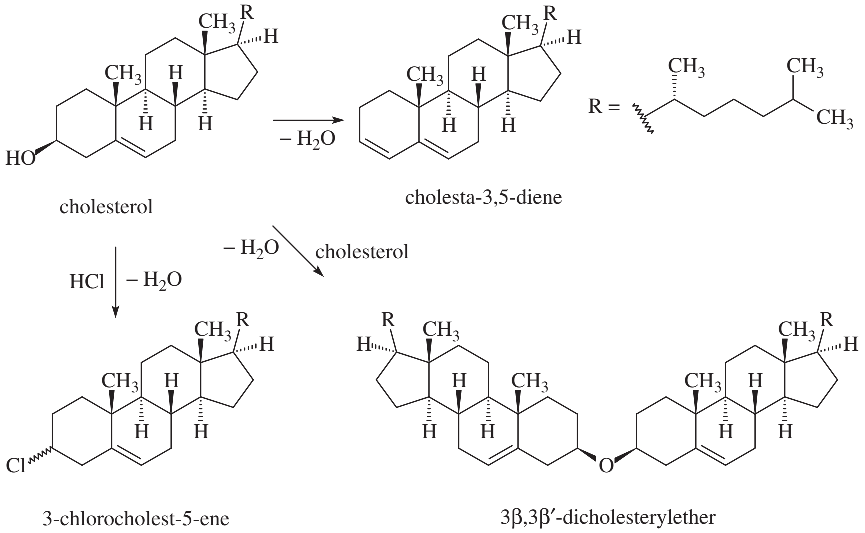


Figure 3.91 Dehydration and other reactions of sterols during heating.

4-methylsterols and to a lesser extent in sterols (4-demethylsterols). Possible reactions are as follows:

- isomerisation of double bonds in the side chain, which gives arise to the geometric and positional isomers of the original steroids;
- hydrogenation of double bonds in the side chain, which leads to steroids with saturated side chains;
- opening of the cyclopropane ring;
- hydrogenation of all double bonds.

The predominant reaction is isomerisation of the double bond in position C-24 (C-28) to position C-24 (C-25) leading to thermodynamically stable isomers. The 4,4-dimethylsterol 24-methylenecycloartanol (**3-105**) yields a mixture of isomers consisting of, among other compounds, cyclosadol (**3-106**) and cyclobranol (**3-107**). Similar isomerisation proceeds in 4-methylsterols such as obtusifoliol (**3-110**) and others (Figure 3.92). The formation of geometric and positional isomers of 4-methylsterols (such as citrastadienol, 3-111) and sterols (such as Δ^5-avenasterol, **3-121**) with ethylidene group in the side chain at C-24 is shown in Figure 3.93. Hydrogenation of double bonds in the side chain yields saturated derivatives. For example, 24-methylenecycloartanol (**3-105**) is transformed to 24-methylcycloartanol and stigmasterol (**3-120**) yields β-sitosterol (**3-119**).

The opening of the cyclopropane ring during hydrogenation occurs in cycloartenol and 24-methylenecycloartanol. The hydrogenation of cycloartenol produces lanosta-9(11),24-dienol (known as parkeol) and lanosterol (lanosta-8,24-dienol). 24-Methylenecycloartanol yields 24-methylenelanost-9(11)-enol and 24-methylenelanost-8-enol. Changes of some steroids in hydrogenated sunflower and soybean oils are documented in Table 3.49.

For food purposes, phytosterols are isolated from tall oil, a byproduct of wood pulp manufacture from coniferous trees. Crude tall oil contains 5–10% of phytosterols that are selectively hydrogenated at the double bond in position C-5 yielding the corresponding **stanols**, the main component of which is sitostanol. It has been shown that if phytosterols (or their fatty acid esters) are consumed at higher levels (e.g. in margarines) they inhibit absorption of exogenous and endogenous cholesterol in the gastrointestinal tract and lower total and LDL cholesterol concentrations, while the usual levels of consumed phytosterols do not significantly affect cholesterol absorption.

3.8.4.3 Oxidation

In vivo (in the biosynthesis of bile acids and some hormones) and also during heat processing and storage of foods, sterols are oxidised in the nuclei and side chain to form various oxygenated derivatives known by the general term **oxysterols**. Some of these compounds are synthesised *in vivo* from cholesterol, but they can also be formed in food during processing procedures and storage. For example,

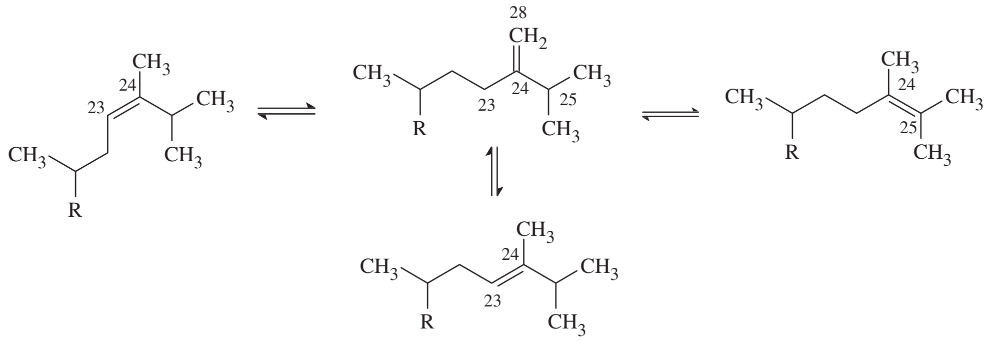


Figure 3.92 Isomerisation of steroids with a methylene group in the side chain (R = steroid residue).

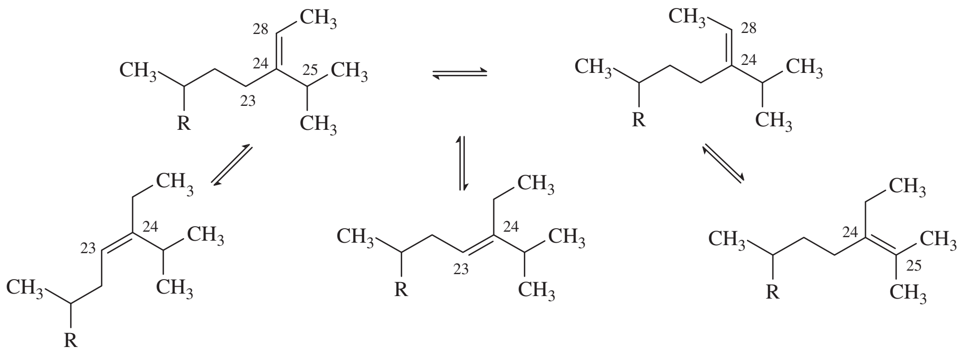


Figure 3.93 Isomerisation of steroids with an ethylidene group in the side chain (R = steroid residue).

Table 3.49 Changes to significant steroids during hydrogenation of vegetable oils.

Steroid	Sunflower oil hydrogenated		Soybean oil hydrogenated	
	Once	Twice	Once	Twice
4,4-Dimethylsterols[a]				
Cycloartenol	35	26	35	26
24-Methylenecycloartanol	25	4	16	0
Cyclobranol	3	18	2	11
24-Methylcycloartanol	2	10	4	9
4-Methylsterols[b]				
Obtusifoliol	25	6	10	2
Gramisterol and cycloeucalenol	12	2	7	2
Citrastadienol	33	15	38	12
Sterols[c]				
Stigmasterol	10	9	17	15
Δ^5-Avenasterol	3	1	1	0.2
Δ^7-Avenasterol	3	2	0.5	0.1

[a]Total content of 4,4-dimethylsterols (triterpenic alcohols) (%).
[b]Total content of 4-methylsterols (%).
[c]Total content of sterols (%).

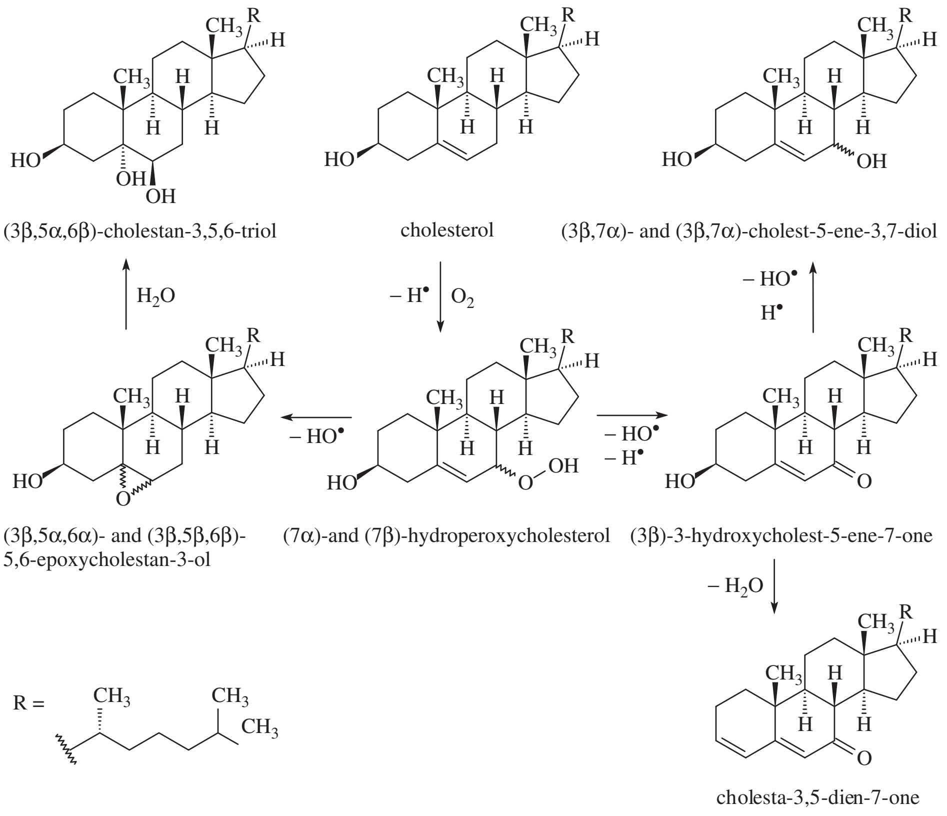


Figure 3.94 Main products of cholesterol oxidation.

Table 3.50 Oxidation products of phytosterols in potato chips fried in sunflower oil.

Phytosterol	Amount of oxidation product (mg/kg)[a]				
	7α-hydroxy-	7β-hydroxy-	7-oxo-	5α,6α- and 5β,6β-epoxy-	5α,6β-dihydroxy-
Sitosterol	4.7	9.7	13.5	5.4	2.8
Campesterol	1.4	1.8	9.2	3.6	1.6

[a] Total amount of phytosterol oxidation products is 53.7 mg/kg.

oxidation of cholesterol by oxygen yields (via a C-7 radical) 7α- and 7β-hydroperoxides. Analogous to the secondary oxidation reactions of fatty acids, hydroperoxides of sterols are transformed into the corresponding 5,6-epoxides (5α,6α-epoxides and 5β,6β-epoxides) and their hydrolysis gives 3,5,6-triols. Alkoxyl radicals formed by decomposition of hydroperoxides give rise to the major cholesterol oxidation products that include epimeric 7-hydroxy derivatives and the 7-oxo derivative, which is further transformed into 3,5-diene-7-one by dehydration (Figure 3.94). In addition to 7-hydroperoxides of cholesterol, epimeric 22-hydroperoxides, 20,25-dihydroperoxides, 5α,6β-dihydroxy derivatives and 2,4,6-trienes have also been identified as oxidation products with triplet oxygen. Oxidation of cholesterol with singlet oxygen produces epimeric 5-hydroperoxides that rearrange to 7-hydroperoxides via the corresponding hydroperoxyl radicals. Phytosterols and steryl esters are oxidised similarly. Plant sterols also undergo oxidative processesses comparable to those involved in cholesterol oxidation.

Sterol oxidation products are found in stored oils and especially in oils used for deep frying, therefore these products are also present in fried potato chips (Table 3.50), fried potato crisps and other fried products. Sterol oxidation products are also found in lipids of dried foods, such as dried milk or eggs.

After absorption, oxysterols react with blood plasma lipoproteins to form complexes that can initiate formation of atherosclerotic deposits in the vascular wall. However, the important oxidation reaction from the physiological point of view is the transformation of provitamins D (ergosterol and 7-dehydrocholesterol) into vitamins D_2 and D_3, respectively.

Reactions of triterpene alcohols are probably related to reactions of sterols, but they have not yet been adequately explored.

4

Saccharides

4.1 Introduction

The term **saccharide** comes from the Greek word 'zahari', meaning sugar. The term is frequently used in chemistry to describe polyhydroxyaldehydes H–[CHOH]$_n$–CH=O and polyhydroxyketones H–[CHOH]$_n$–C(=O)–[CHOH]$_m$–H, which contain three or more aliphatic carbon atoms in the molecule. This term also includes derived substances that are formed from these three carbon compounds by condensation reactions with the formation of acetal bonds, and includes monosaccharides, oligosaccharides and polysaccharides, as well as substances derived from saccharides through the reduction of the carbonyl group, the oxidation of one or more terminal groups or the replacement of one or more hydroxy group(s) by a hydrogen atom, an amino group, a thiol group or similar heteroatomic groups. The term **carbohydrate** is actually a descriptor of what these molecules are theoretically composed of. It was applied originally to monosaccharides, in recognition of the fact that they are carbon hydrates in a ratio of one carbon molecule to one water molecule, so that their empirical composition can be expressed as $C_n(H_2O)_n$. However, the term is now used generically in a wider sense, and also includes derivatives of these compounds. The term carbohydrate is most common in biochemistry, medicine and nutrition. The former name, 'glycides', is now used less frequently and is not recommended for use in chemistry.

Depending on the number of carbon atoms in the molecule, saccharides are divided to **trioses**, **tetroses**, **pentoses**, **hexoses** and higher sugars. Compounds with aldehyde functional group are called **aldoses** (e.g. aldopentoses and aldohexoses) and compounds with a ketone function are called **ketoses** (such as ketohexoses).

Depending on the number of sugar units bound in the molecule, saccharides are divided into:

- **monosaccharides**
- **oligosaccharides**
- **polysaccharides** also known as **glycans**
- **complex** or **conjugated saccharides**.

Monosaccharides consist of only one sugar unit. Oligosaccharides consist of between two and ten of the same or different monosaccharides connected to each other by glycosidic (semiacetal) bonds. Monosaccharides and oligosaccharides are sometimes referred to collectively by the name sugars, as they share many common properties and often have a sweet taste. Polysaccharides are composed of more than ten of the same or different monosaccharides; commonly they consist of multiple molecules of monosaccharides, which are often precisely determined. Complex carbohydrates also contain compounds other than saccharides, such as, for example, peptides, proteins and lipids.

Saccharides arise naturally in cells of photoautotrophic organisms (green plants and photosynthetic bacteria) by assimilation of carbon dioxide in the air in the presence of water and energy, and by use of natural light (photosynthesis) converted by photosystems into chemical energy. Heterotrophic organisms obtain the necessary saccharides from autotrophic organisms or from non-saccharide carbon substrates, such as certain amino acids, hydroxy acids, glycerol and other substances (the metabolic pathway is called gluconeogenesis), or they transform saccharides into different structures. Saccharides are common components of all cells. The level of saccharides in animal tissues is only a few percent, but in plant tissues they commonly represent 85–90% of dry matter.

Saccharides have various functions in cells:

- They are used primarily as an energy source (e.g. polysaccharides, oligosaccharides and monosaccharides), 1 g glucose provides 17 kJ (4 kcal), the energy yield of sugar alcohols is only 10 kJ/g (2.4 kcal/g) and are therefore main (primary) nutrients together with proteins and lipids.
- They are the basic building units of many cells, and protect cells against the effects of various external factors (e.g. some oligosaccharides, polysaccharides and complex saccharides).

The Chemistry of Food, First Edition. Jan Velíšek.
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- They are biologically active substances (e.g. oligosaccharides in milk) or components of many biologically active substances such as glycoproteins, some coenzymes, hormones and vitamins.

Saccharides are highly reactive substances that are transformed into many different products, even without the participation of other reaction partners, during food storage and processing. The most common and important reactions of carbohydrates are their reactions with amino compounds that are known as non-enzymatic browning reactions, which also involve reactions of carbohydrates in the absence of amino compounds. Reactions of carbohydrates with amino compounds (proteins, amino acids) are the Maillard reactions. The products of these reactions are important flavour-active compounds and are yellow, brown or black pigments in many foods and compounds with beneficial properties (e.g. antioxidants), but can also be substances with antinutritional or even toxic effects.

This chapter on saccharides is divided into two main parts. The first part describes major monosaccharides, and their functional derivatives, oligosaccharides and polysaccharides. Their structure and nomenclature, occurrence in major food commodities, properties and importance in human physiology and nutrition, recommended intake and use in food technology are all described. The second part is devoted to reactions of saccharides that lead to the formation of products that influence odour, taste and colour of foods, and to reactions taking place during storage and thermal processing of food raw materials and foods.

4.2 Monosaccharides

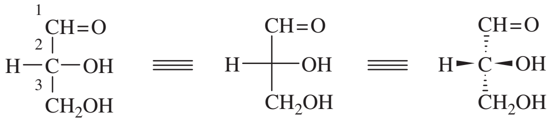
4.2.1 Structure and nomenclature

Monosaccharides, aldoses and ketoses, are divided – according to the number of carbons in the chain – into trioses, tetroses, pentoses, hexoses and higher sugars, and into aldotrioses, ketotrioses and so on. The carbon chain of monosaccharides present in foods is usually linear, but branched-chain monosaccharides also exist.

Monosaccharides occur as substances with a free carbonyl group (acyclic compounds) and as cyclic **hemiacetals**, also called **lactols**. Trioses are exclusively acyclic substances, tetroses and higher monosaccharides exist predominantly in five- and six-membered and, exceptionally, also in seven-membered cyclic structures. They can therefore be regarded as substances derived from oxolane (tetrahydrofuran), oxane (tetrahydropyran) or oxepane, and are thus actually heterocyclic compounds. Acyclic forms, which exist in constitutional equilibrium with cyclic forms, occur in zigzag conformers, as well as alditols (see Section 4.3.1.1.1).

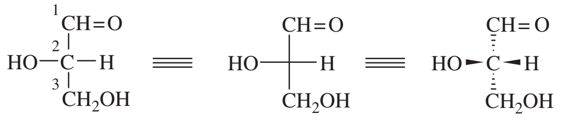
4.2.1.1 Aldoses

The simplest aldose is aldotriose, known as glyceraldehyde, which contains the aldehyde group in position C-1 and a chiral carbon atom in position C-2. It exists therefore in two ($2^1 = 2$; generally 2^n, where n is the number of chiral carbon atoms) configuration isomers, as D-glyceraldehyde (**4-1**) and L-glyceraldehyde



4-1, D-glyceraldehyde (D-*glycero*-triose)

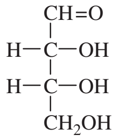
(**4-2**). Solutions of D-glyceraldehyde rotate the plane of polarised light to the right (clockwise) and D-glyceraldehyde is therefore (+)-glyceraldehyde or (+)-D-glyceraldehyde or d-glyceraldehyde (is dextrorotary). L-Glyceraldehyde solutions rotate the plane of polarised light to the left (counter clockwise) therefore L-glyceraldehyde is (−)-glyceraldehyde or (−)-L-glyceraldehyde or l-glyceraldehyde (is laevorotary).



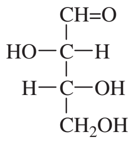
4-2, L-glyceraldehyde (L-*glycero*-triose)

Isomers are called **optical isomers**, **antipodes** or **enantiomers**. Their equimolar mixture is optically inactive and is called a **racemate**. Symbols (affixes) *R* and *S* are used only rarely for labeling configurations of saccharides. D-Glyceraldehyde is thus (*R*)-glyceraldehyde and L-glyceraldehyde is (*S*)-glyceraldehyde. The former name for glyceraldehyde was glycerose. In biochemistry, the simplest two carbon sugar glycolaldehyde (biose) and the simplest one carbon sugar formaldehyde (monose) are also classified as saccharides (aldoses).

The prefixes D- and L- are also used with other monosaccharides. The formal insertion of clusters of atoms H–C–OH (or the mirror image of HO–C–H) between the first and second carbons of a D-glyceraldehyde molecule gives two optically active aldotetroses called D-erythrose (**4-3**) and D-threose (**4-4**). L-Glyceraldehyde can yield L-erythrose (**4-5**) and L-threose (**4-6**) in the same way. The total number of **configuration isomers** (**stereoisomers**) is $2^2 = 4$. The pairs of saccharides D-erythrose with L-erythrose as well as D-threose with L-threose are **enantiomeric** substances (asymmetric carbons have the opposite configuration; one substance is a mirror image of the other). The pair from D-erythrose with D-threose as well as the pair from L-erythrose with L-threose are **diastereoisomeric** substances that differ in configuration at one chiral centre, while the configuration at the second chiral centre is identical.

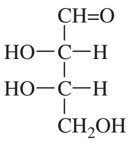


4-3, D-erythrose
(D-*erythro*-tetrose)

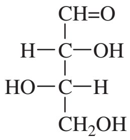


4-4, D-threose
(D-*threo*-tetrose)

In a similar way, a total of eight optically active linear aldopentoses (four pentoses of the D-series and four pentoses of the L-series) and a total of 16 linear aldohexoses

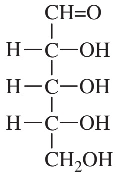


4-5, L-erythrose
(L-*erythro*-tetrose)

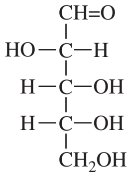


4-6, L-threose
(L-*threo*-tetrose)

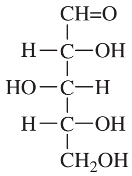
(eight hexoses of the D-series and eight hexoses of the L-series) can be derived. Structures of D-aldopentoses and D-aldohexoses in Fischer projections are given in formulae **4-7** to **4-10** and **4-11** to **4-18**.



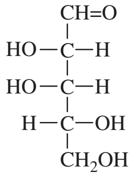
4-7, D-ribose
(D-*ribo*-pentose)



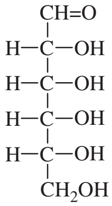
4-8, D-arabinose
(D-*arabino*-pentose)



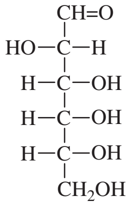
4-9, D-xylose
(D-*xylo*-pentose)



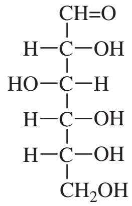
4-10, D-lyxose
(D-*lyxo*-pentose)



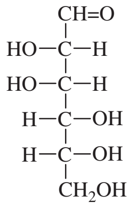
4-11, D-allose
(D-*allo*-hexose)



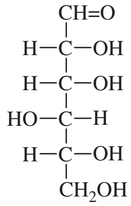
4-12, D-altrose
(D-*altro*-hexose)



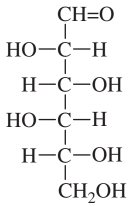
4-13, D-glucose
(D-*gluco*-hexose)



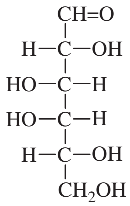
4-14, D-mannose
(D-*manno*-hexose)



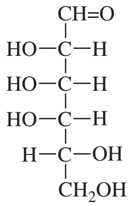
4-15, D-gulose
(D-*gulo*-hexose)



4-16, D-idose
(D-*ido*-hexose)



4-17, D-galactose
(D-*galacto*-hexose)

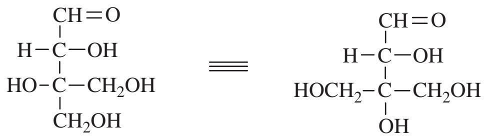


4-18, D-talose
(D-*talo*-hexose)

The affiliation of an aldose to the D- or L-series is determined by the conformity of the configuration of the chiral carbon atom with the highest sequence number in the molecule (such as carbon C-3 in aldotetroses, carbon C-4 in aldopentoses and carbon C-5 in aldohexoses), with the configuration of the chiral carbon atom of either D-glyceraldehyde or L-glyceraldehyde. Whether the aldose belongs to the D- or L-series of sugars is independent of the configuration of the other carbon atoms. For example, (+)-D-glucose configuration at carbons C-5, C-4 and C-2 is D (*R* configuration) and the carbon C-3 has an L configuration (*S* configuration). The change in configuration at the other chiral atom provides a different aldose of the same series (e.g. changing the configuration at C-4 of D-glucose gives D-idose). The change in configuration of all chiral atoms, in the case of the D-isomer, gives the corresponding L-isomer. For example, D-glucose is transformed into L-glucose and vice versa. Aldoses, which differ only in configuration at C-2 are called **epimers**. Examples of epimers are D-glucose and D-mannose.

Removing the suffix '-se' from the trivial names of trioses, tetroses, pentoses and hexoses gives the configuration prefixes *glycero*-, *erythro*-, *threo*-, *ribo*-, *arabino*-, *xylo*-, *lyxo*-, *allo*-, *altro*-, *gluco*-, *manno*-, *gulo*-, *ido*-, *galacto*- and *talo*-, which are, together with the configuration symbols D and L, the basis of systematic carbohydrate nomenclature. The systematic name of D-glyceraldehyde is then D-*glycero*-triose, D-erythrose is D-*erythro*-tetrose, D-ribose is D-*ribo*-pentose and D-glucose is D-*gluco*-hexose. For practical reasons, however, the preferred names are trivial names, if the saccharide structure is expressed uniquely.

In addition to sugars with straight chains, branched chain sugars also exist in nature, for example as components of pectin and other polysaccharides. Again, their preferred names are the trivial names. Aldopentose D-apiose with one chiral carbon atom has the systematic name 3-*C*-hydroxymethyl-D-*glycero*-tetrose (**4-19**).



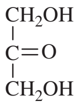
4-19, D-apiose

The abbreviated notations of monosaccharides are formed as they are for amino acids. An abbreviation of the monosaccharide name consists of the first three letters of the trivial name. An exception, however, is glucose, for which the abbreviation Glc is used as the symbol Glu is reserved for glutamic acid.

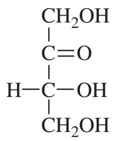
4.2.1.2 Ketoses

The simplest ketose (ketotriose) is optically inactive 1,3-dihydroxyacetone, also known as 1,3-dihydroxypropan-2-one or glycerone (**4-20**). Similar to aldotetroses and higher aldoses derived from glyceraldehyde, one D-ketotetrose (**4-21**), two D-ketopentoses (**4-22**, **4-23**), four D-ketohexoses (**4-24** to **4-27**), and the same number of ketoses of the L-series, can be derived from 1,3-dihydroxyacetone. Trivial names are used for ketohexoses, but for higher ketoses systematic names are preferred. Ketoses with more than four chiral centres are formally divided into two parts so that the four chiral carbon atoms have the lowest serial numbers. The name starts from the centre with the highest number. Ketose **4-28**, for example, is called D-*manno*-hept-2-ulose, D-*altro*-hept-2-ulose is called trivially sedoheptulose,

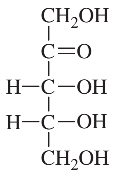
sugar **4-29** is D-*glycero*-D-manno-oct-2-ulose and sugar **4-30** is D-*erythro*-L-*gluco*-non-2-ulose. A branched ketohexose with the trivial name dendroketose (**4-31**) has the systematic name D-4-deoxy-4-*C*-hydroxymethyl-D-*glycero*-pent-2-ulose.



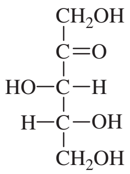
4-20, 1,3-dihydroxyacetone



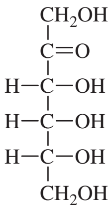
4-21, D-erythrulose (D-*glycero*-tetrulose)



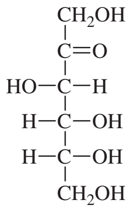
4-22, D-ribulose (D-*erythro*-pent-2-ulose)



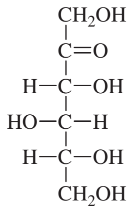
4-23, D-xylulose (D-*threo*-pent-2-ulose)



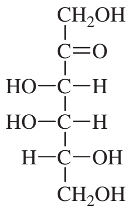
4-24, D-psicose (D-*ribo*-hex-2-ulose)



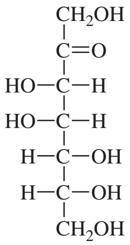
4-25, D-fructose (D-*arabino*-hex-2-ulose)



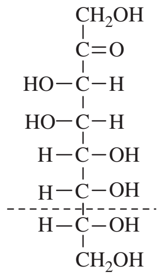
4-26, D-sorbose (D-*xylo*-hex-2-ulose)



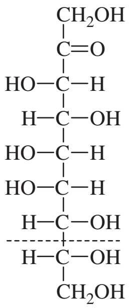
4-27, D-tagatose (D-*lyxo*-hex-2-ulose)



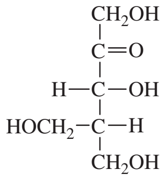
4-28, D-*manno*-hept-2-ulose



4-29, D-*glycero*-D-*manno*-oct-2-ulose



4-30, D-*erythro*-L-*gluco*-non-2-ulose



4-31, D-dendroketose

4.2.1.3 Cyclic structures

Aldehydes and ketones react with alcohols to form unstable hemiacetals. A reaction of hemiacetal with another molecule of alcohol yields stable acetals (see Figure 8.22). In contrast, saccharides spontaneously yield only stable cyclic hemiacetal by intramolecular addition of one hydroxyl group (primary or secondary hydroxyl group) to the carbonyl group. These cyclic forms of aldoses and ketoses are generally called **lactols**. Six membered or five membered (and exceptionally seven membered) rings are mainly formed. Structures with five membered rings are called **furanoses**, the six membered rings are **pyranoses**, and the seven membered rings are **septanoses**.

On the carbon of the carbonyl group (C-1 carbon of aldoses and C-2 carbon of ketoses), a new chiral centre arises due to the ring formation. The carbon of the carbonyl group is called an **anomeric carbon**, the newly formed hydroxyl group at the anomeric carbon is an **anomeric hydroxyl group** (hemiacetal group) and the corresponding pairs of isomers are **anomers**. To mark the configuration of substituents on the anomeric carbon, configuration prefixes α and β, indicating the relative configuration to the chiral carbon atom with the highest number (which determines the affiliation of the sugar to the D- or L-series) are used. The α anomer has the same configuration while the β anomer has the opposite configuration. Both anomers, which are special types of diastereoisomers, differ in their optical rotations.

The formation of cyclic structures from acyclic structures and the relationship between different types of formulae (Fisher, Tollens, Haworth and Mills projection formulae) are shown in Figure 4.1 for the example of D-glucose. In the Fischer representation of a monosaccharide, the carbon chain is written vertically, with the lowest numbered carbon atom at the top and the neighbouring carbon atoms below. The H and OH groups projects to the right or the left. A monosaccharide is assigned to the D or the L series according to the configuration at the highest-numbered centre of chirality. A sugar that belongs to the D series has the highest-numbered hydroxy group projected to the right in the Fischer projection. If a cyclic form of a sugar is to be represented by the Tollens formula in the Fischer projection, a long bond can be drawn between the oxygen involved in ring formation and the (anomeric) carbon atom to which it is linked. Cyclic forms of D-glucose and D-fructose can be represented by formulae **4-32** to **4-39**. Conventionally, the formula in the Haworth projection is drawn so that the planar heterocyclic ring lies perpendicular to the picture plane and the oxygen atom is behind the plane. Axial and equatorial positions are not distinguished; instead, substituents are positioned above or below the ring atom to which they are connected and they are directed above or below the plane, if they lie in the Fischer projection on the left or the right side of the formula. The ring part directed to the front of the plane is usually drawn in bold. Formulae can rotate or flip in space if necessary. In some cases, formulae can be clarified by use of the Mills depiction, in which the main hemiacetal ring is drawn in the plane of the paper; dashed bonds denote substituents below this plane, and thickened bonds those above.

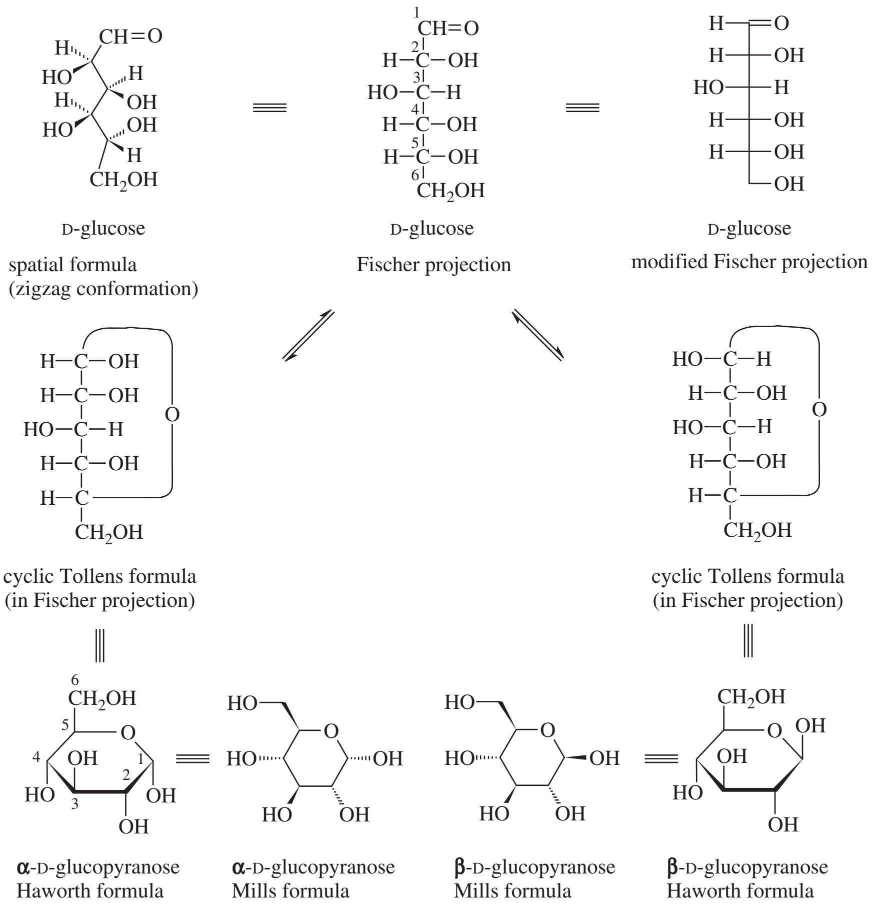
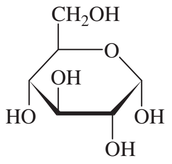
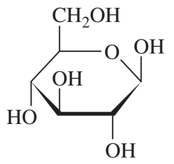


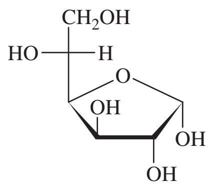
Figure 4.1 Acyclic and cyclic forms of D-glucose.



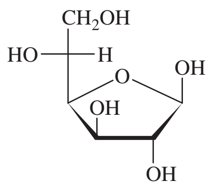
4-32, α-D-glucopyranose



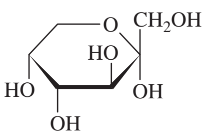
4-33, β-D-glucopyranose



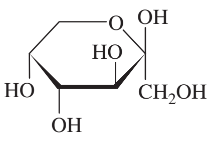
4-34, α-D-glucofuranose



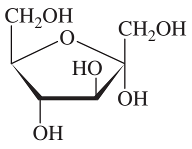
4-35, β-D-glucofuranose



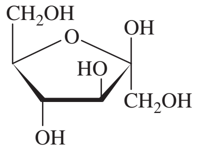
4-36, α-D-fructopyranose



4-37, β-D-fructopyranose



4-38, α-D-fructofuranose



4-39, β-D-fructofuranose

4.2.1.3.1 Mutarotation

In the crystalline state, reducing monosaccharides exist exclusively in cyclic structures (α- or β-anomers). In solutions, a balance between the α- and β-anomers and acyclic forms is established as the individual forms interconvert over time. This process, schematically shown in Figure 4.2, is called **mutarotation**. The mechanism of mutarotation assumes cleavage of the saccharide cyclic forms to neutral acyclic saccharide or its ion that have free carbonyl groups. The equilibrium composition of the mixture (the amount of pyranoses, furanoses or septanoses) depends on the type of sugar, solvent, pH (the reaction generally involves acid–base catalysis) and temperature.

Crystalline D-glucose, existing as an anhydrous substance or monohydrate, is α-D-glucopyranose. In weakly acidic and neutral aqueous solutions at ambient temperature, the establishment of

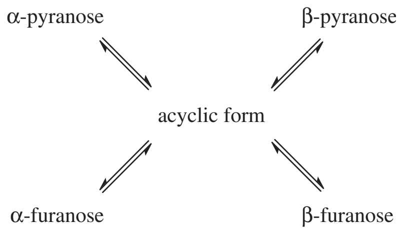


Figure 4.2 Mutarotation of monosaccharides.

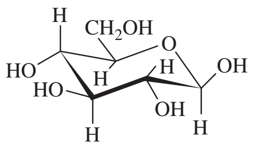
equilibrium takes for about 4 h and in weakly alkaline solutions the equilibrium is established immediately. The equilibrium aqueous solution at 20 °C contains about 33% of α-D-glucopyranose and 67% of β-D-glucopyranose; the solution at 40 °C contains 36% of the α-anomer and 64% of the β-anomer. Furanose forms of glucose are present at levels of up to 1%. The composition of the equilibrium solutions of ordinary aldoses and fructose are given in Table 4.1. In aqueous solutions (at 20 °C), the acyclic form of D-glucose is present at levels of 0.02–0.06%. Aqueous solutions of other aldohexoses and ketohexoses contain similar amounts of acyclic forms. Solutions of pentoses contain higher levels of acyclic forms than hexoses (e.g. D-ribose, 8.5%). Trioses are found exclusively in the acyclic forms. In enzymatically active plant and animal material, the mutarotation of saccharides containing bound glucose and galactose is catalysed by mutarotase (aldose 1-epimerase).

4.2.1.3.2 Conformation

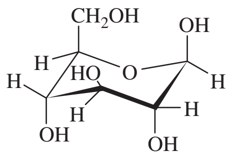
Pyranoses

Cyclic forms of saccharides are planar structures. The thermodynamically most favourable and therefore most common conformations of pyranoses are chair (C) conformations. Boat (B), skew (S) and half-chair (H) conformations are rarely found.

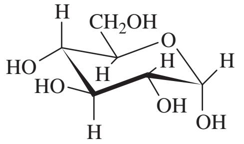
Each pyranose anomer can exist in two chair structures, $^4C_1$ and $^1C_4$. Numbers 1 and 4 correspond to atoms C-1 and C-4, which are above (expressed as superscript) or structures below (expressed as subscript) of the reference plane defined by carbons C-2, C-3, C-5 and the oxygen atom. As an example, structures of both chair conformers of β-D-glucopyranose and α-D-glucopyranose are given in formulas **4-40** to **4-43**.



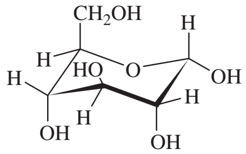
4-40, β-D-glucopyranose-$^4C_1$



4-41, β-D-glucopyranose-$^1C_4$



4-42, α-D-glucopyranose-$^4C_1$



4-43, α-D-glucopyranose-$^1C_4$

In solutions, conformers $^4C_1$ and $^1C_4$ of saccharides are in equilibrium in which the predominant conformer has a larger number of substituents (hydroxyl or hydroxymethyl groups) in the equatorial positions, because (except for the substituent on the anomeric carbon atom) bulky axial substituents are thermodynamically unfavourable. Predominantly **1,3-*syn*-axial** interactions of hydroxyl and hydroxymethyl groups take place, such as, for example, interactions of the hydroxyl group in position C-4 and the hydroxymethyl group in conformer **4-41**. The hydroxymethyl group as well as the anomeric hydroxyl of aldohexoses are always equatorial, which means that D-aldohexoses prefer the conformation $^4C_1$. From the conformational point of view, the most stable aldohexose is the $^4C_1$ conformer of β-D-glucopyranose because all five bulky substituents are in equatorial positions (Table 4.2). An aqueous solution of glucose, however, contains only about 64% of this anomer and the rest is α-anomer. The reason is that the stability of the axial hemiacetal hydroxyl group of α-D-glucopyranose-$^4C_1$ (**4-42**) is increased due to the **anomeric effect** manifested by the interaction with the oxygen atom of the pyranose ring, through electrostatic repelling of their non-bonding electrons. If the hydroxyl group is in the equatorial position (β-anomer), the angle between the dipole moments is small and electrostatic repulsive forces are large. If the hydroxyl group is in the axial position, the repulsive forces are smaller. The axial position is therefore preferable.

Table 4.1 Cyclic forms of some hexoses in aqueous solutions at 40 °C.

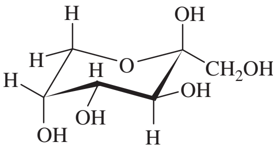
	Furanose (%)		Pyranose (%)			Furanose (%)		Pyranose (%)	
Saccharide	α	β	α	β	Saccharide	α	β	α	β
D-Ribose	6	18	20	56	D-Gulose	<1	<1	21	79
D-Allose	5	7	18	70	D-Idose	16	16	31	37
D-Altrose	20	13	28	39	D-Galactose	<1	<1	27	73
D-Glucose	<1	<1	36	64	D-Talose	<1	<1	58	42
D-Mannose	<1	<1	67	33	D-Fructose	<1	25	8	67

Table 4.2 Position of substituents (e = equatorial, a = axial) in $^4C_1$ conformers of aldohexoses.

	Hydroxyl group				Hydroxyl group		
Saccharide	C-2	C-3	C-4	Saccharide	C-2	C-3	C-4
D-Allose	e	a	e	D-Gulose	e	a	a
D-Altrose	a	a	e	D-Idose	a	a	a
D-Glucose	e	e	e	D-Galactose	e	e	a
D-Mannose	a	e	e	D-Talose	a	e	a

The 1,3-diaxial interaction between a C-1 hydroxyl group and the axial protons at C-3 and C-5 also operates in favour of β-anomers. The resulting effect is that about 36% of D-glucose in equilibrium is present in the form of the α-anomer (at 20 °C). The conformation of glucose molecules explains the almost universal role of glucose in living systems as a structural unit of many biopolymers and an intermediate in a number of important reactions.

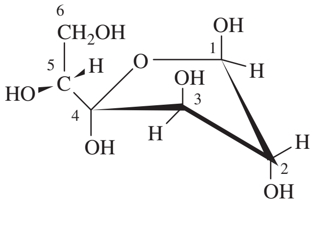
D-Fructose is found primarily as β-pyranose in conformation $^1C_4$ in which the hydroxyl groups at C-3 and C-4 are in the equatorial positions and the hydroxyl group at C-5 is in the axial position (**4-44**).



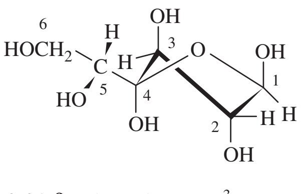
4-44, β-D-fructopyranose-$^1C_4$

Furanoses

Furanose conformers differ from each other in their energy only slightly and can interconvert between the different conformations very quickly. The most frequently occurring are envelope (E) and twist (T) conformations. Both of these basic conformations have almost equal energy. In fact, there are ten E conformations and ten T conformations that interconvert. The interconversion between the many conformers is called **pseudorotation**. Formulae **4-45** and **4-46** are examples of E and T conformers of β-D-glucofuranose. The type of conformer and levels of anomers in furanose solutions and in foods depends on the intramolecular non-bonding interactions.



4-45, β-D-glucofuranose-E_2



4-46, β-D-fructofuranose-$^3T_2$

4.2.2 Occurrence

Monosaccharides are common components of almost all foods, but their content is highly variable, as is the representation of individual sugars. The most common monosaccharides in foods are hexoses and pentoses.

Monosaccharides are present in relatively large quantities in fruits, where their content increases during ripening. However, this varies greatly depending on the type of fruit, the level of maturity, the conditions of post-harvest storage, processing and so on. In apples, for example, only traces of starch are present at the time of harvest, and during post-harvest ripening, this starch is completely degraded. A partial decomposition of hemicelluloses and pectin also occur, and the monosaccharides content rises.

D-Glucose (**4-13**), also known as dextrose, grape sugar or starch sugar, is, together with D-fructose (**4-25**), known as laevulose or fruit sugar, the main monosaccharide of most foods. In the blood of animals, it occurs at concentrations of about 1 g/kg, in diabetic patients (*diabetes mellitus*) it may occur in the urine, in extreme cases at concentrations up to 10%. Free D-mannose (**4-14**), D-galactose (**4-17**) and other hexoses and their derivatives are found in the small quantities in a variety of foods. Pentoses in foods are generally present in smaller amounts than hexoses. The main pentoses are D-ribose (**4-7**), L-arabinose (**4-8**) and D-xylose (**4-9**), which is otherwise known as wood sugar.

Saccharides are deliberately added to a variety of food products to improve their organoleptic properties (taste, texture). Monosaccharides are usually added as invert sugar and in the form of glucose or fructose syrups.

4.2.2.1 Meat and meat products

Glycogen (also known as animal starch) occurs in the muscles of warm-blooded animals in concentrations ranging from 0.02 to 1% (0.3% in fish), depending on age and other factors, and is rapidly degraded post-mortem. In the matured flesh, only monosaccharides and their phosphoric acid esters occur. They are commonly present at levels of 0.1–0.15%, of which glucose 6-phosphate constitutes about 0.1% and about a fifth of this amount represents (0.02%) glucose 1-phosphate and fructose 1,6-bisphosphate. The rest is mainly glucose (0.009–0.09%), fructose and ribose. Ribose arises primarily as a hydrolysis product of free nucleotides (NAD, NADP, ATP and the corresponding nucleosides). A certain proportion of ribose is present in the form of ribitol in riboflavin (vitamin B_2) and other flavins.

4.2.2.2 Milk and dairy products

Monosaccharides, particularly glucose, are present in milk in insignificant quantities. The main sugar is disaccharide lactose, while other related oligosaccharides occur in milk in smaller amounts.

4.2.2.3 Eggs

The amount of saccharides in eggs (in dry matter) is about 10 g/kg. About 9 g/kg of sugars are present in egg white and 1 g/kg in

Table 4.3 Basic composition of honey.

Constituent	Average content (%)	Range	Constituent	Average content (%)	Range
Water	17.2	13.4-22.9	Fructose	38.2	27.3-44.3
Proteins (enzymes)	0.4	0.1-0.6	Saccharose	1.3	0.3-7.6
Minerals	0.17	0.02-1.03	Maltose	7.3	2.7-16.0
Glucose	31.3	22.0-40.8	Higher sugars	1.5	0.1-8.5

the yolk. Protein-bound saccharides in the form of glycoproteins occur in the egg white at a level of about 5 g/kg, and in the yolk at about 0.2 g/kg. The rest are free sugars, especially monosaccharides, but free oligosaccharides and polysaccharides do not occur in the egg white. About 98% of free monosaccharides are glucose, while mannose, galactose, arabinose, xylose, ribose and 2-deoxyribose (2-deoxy-D-*erythro*-pentose) are present at concentrations of 2–20 mg/kg. In the protein-bound carbohydrates, galactose, mannose, glucosamine, galactosamine and lactaminic acid predominate.

4.2.2.4 Honey

The main sugar components of honey are glucose, fructose and maltose (Table 4.3). To a lesser extent there are also a number of various oligosaccharides present, whose composition is given later in Table 4.12.

4.2.2.5 Cereals and cereal products

Mono-, di-, tri- and higher oligosaccharides resulting from the degradation of starch are present in low concentrations in cereals. Wheat flour contains 100–900 mg/kg glucose and 200–800 mg/kg fructose. The maltose content is 500–1000 mg/kg, saccharose and raffinose occur in amounts of 1000–4000 mg/kg and 500–1700 mg/kg, respectively, and the amounts of other oligosaccharides ranges from 0.4 to 1.6%. The sugars content in cereal products is highly variable as it primarily depends on the degree of starch hydrolysis or the amount of added saccharides.

4.2.2.6 Fruits and vegetables

The main sugars in fruits are glucose (about 0.5–32%) and fructose (about 0.4–24%), and other monosaccharides are present to a lesser extent (Table 4.4). Ripe grapes, for example, contain glucose and fructose in roughly the same amounts (about 8%), but fructose dominates in overripe grapes. The sugar content in wine musts is 120–250 g/l (expressed as glucose). In addition to glucose and fructose (their ratio is 0.5–0.9), wines contain relatively large quantities of arabinose, xylose, galactose and small amounts of other monosaccharides and oligosaccharides (Table 4.5). According to the sugar content, wines are categorised as dry wines (up to 4 g of residual sugar per liter), semi-dry wines (4.1–12 g/l),

Table 4.4 Content of monosaccharides and saccharose in fresh fruits (% of edible portion).

Fruits	Water	Glucose	Fructose	Saccharose
Apple	84.0	1.8-2.6	5.0-8.7	2.0-2.4
Apricot	87.4	1.9	0.4	4.4
Banana	73.6	5.6-5.8	3.5-3.8	6.6-13.9
Black currant	80.3	2.4	3.7	0.6
Blueberry	84.2	4.8	4.9	0.0
Cherry	81.3	5.5	6.1	0.0
Date	20.0	32.0	23.7	8.2
Fig	78.0	5.5	4.0	0.0
Grapefruit	88.6	2.0-2.7	1.2-2.7	2.1-2.2
Grape[a]	82.7	8.2-9.7	8.0-11.4	0.0-0.03
Kiwifruit	83.1	4.8	5.3	0.7
Lemon	88.3	0.5	0.5-0.9	0.1-0.2
Mandarin	85.2	2.2	2.4	0.9
Mango	83.5	0.3	2.5	4.5
Orange	87.0	1.8-2.4	1.9-2.4	4.7
Peach	87.1	0.7-1.5	0.9-1.1	6.7-9.6
Pear	82.5	1.4-2.2	6.0-11.2	0.3-1.1
Pineapple	84.6	2.3-3.5	1.4-3.9	1.0-7.9
Plum	86.0	3.5	1.3	1.5
Raspberry	86.1	2.2-2.3	2.4-2.5	0.0-1.0
Red currant	83.6	2.3-3.7	1.0-4.5	0.0-0.2
Strawberry	89.8	1.8-2.6	2.1-2.3	1.3-1.7

[a] The content of saccharose in varieties of common grape vine (*Vitis vinifera*, Vitaceae) is low, but in some varieties of Fox grape vine (*V. labrusca*), originating from North America, and their hybrids it may account for up to 25% of total sugars.

semi-sweet wines (12.1–45 g/l) and sweet wines (minimum of 45 g residual sugar). Sparkling wines (champagne wines) are divided into brut nature (naturally dry, sugar content is lower than 3 g/l, where sugar has not been added), extra brut (particularly

Table 4.5 Saccharides content of wines.

Saccharides	Content (mg/l)	Saccharides	Content (mg/l)
Monosaccharides		Fucose	2–9
Ribose	6.3–62	***Oligosaccharides***	
Arabinose	1.0–242	Trehalose	0–61
Xylose	0.6–146	Cellobiose	2–7
Glucose	56–25 000	Maltose	1–5
Mannose	2–37	Saccharose	0
Galactose	6.3–249	Lactose	1–5
Fructose	93–26 500	Melibiose	traces–1
Rhamnose	2.2–121	Raffinose	0–1

dry, 0–8 g/l), brut (dry, <15 g/l, extra dry (especially dry, 12–20 g/l, sec (dry, 17–35 g/l), demi-sec (half dry, 33–50 g/l) and doux (sweet, >50 g/l). The galactose content of fresh fruits ranges from about 11 mg/kg in strawberries to 70–160 mg/kg in grapes and 270 mg/kg in kiwis. Some fruits contain large amounts of less common sugars. Rowanberry wine, for example, contains L-sorbose, which is formed by oxidation of D-glucitol on carbon C-5 during fermentation. Higher ketoses (heptuloses) occur in small quantities in strawberries, grapes and other fruits and in wines. Avocado has a particularly interesting composition of sugars consisting of a number of heptuloses, octuloses and nonuloses (0.2–5% of fresh weight), such as D-*manno*-hept-2-ulose (4-28), D-*talo*-hept-2-ulose, D-*glycero*-D-*manno*-oct-2-ulose (**4-29**), D-*glycero*-L-*galacto*-oct-2-ulose, D-*erythro*-L-*gluco*-non-2-ulose (**4-30**), D-*erythro*-L-*galacto*-non-2-ulose and other sugars.

The main monosaccharides in vegetables, as well as in fruits, are glucose and fructose (Table 4.6). Other monosaccharides (such as galactose, arabinose and xylose) are present at low levels. The galactose content in fresh vegetables ranges from about 22–26 mg/kg in butter lettuce to 33–108 mg/kg in potatoes and 140–400 mg/kg in red bell peppers. Free arabinose occurs in larger quantities in a dried byproduct, known as dried beet pulp, which is left after most of the sugar has been extracted from the sliced beets.

Hydrolysis of saccharose in harvested potato tubers results in the production of glucose and fructose. Changes in the reducing sugar level can be used as an indicator of stress (such as low storage temperature and physical damage) in tubers. For example, tubers stored at low temperature have a sweet taste and will produce French fries of darker colour.

4.2.2.7 Other foods

In legumes, the main monosaccharides are glucose and fructose. In beans, the content of glucose is 0.1–1.1% (fresh weight), around 0.3% in peas and in soybeans it varies from 0.04 to 0.2%. Fructose content in beans is 0.1–1.2%, in peas and soybeans about 0.2 and 0.5–3.2%, respectively. Saccharose and other oligosaccharides are present in higher amounts in legumes (see Table 4.16).

The monosaccharides and sugar alcohols content of higher fungi is generally less than 1% of dry matter. The main monosaccharide is glucose, but the sugar alcohols content is generally higher.

Glucose, fructose, mannose, galactose and other monosaccharides are the building blocks of many oligosaccharides, polysaccharides and heteroglycosides. A less common monosaccharide is apiose (**4-19**), which is an important component of pectin and in the form of glycosides occurs in vegetables of the Apiaceae family, commonly known as the carrot or parsley family, where the main carbohydrate starch is found in the root vegetables and root crops. Some gourmet vegetables belonging to the Asteraceae family, such as globe artichoke (*Cynara cardunculus*), common chicory (*Cichorium intybus*) and black salsify (*Scorzonera hispanica*), contain, in addition to starch, as a reserve polysaccharide inulin, composed of fructose units. Other main polysaccharides are, as in fruits, cellulose, hemicelluloses and pectin.

Table 4.6 Main carbohydrate content of fresh vegetables (% of edible portion).

Vegetable	Water	Glucose	Fructose	Saccharose	Vegetables	Water	Glucose	Fructose	Saccharose
Artichoke	84.9	0.02	0.01	0.02	Eggplant	92.3	0.96	0.83	0.15
Beetroot	87.6	0.22	0.14	10.70	Endive	93.8	0.83	0.59	0.03
Broccoli	89.0	0.49	0.68	0.10	Lettuce (iceberg)	95.6	0.49	0.39	0.02
Cabbage	92.2	0.69	0.61	0.25	Onion	89.1	1.54	1.76	0.22
Carrots	88.3	0.59	0.55	3.59	Pepper	93.9	1.16	1.12	0.11
Cauliflower	91.9	0.58	0.70	0.15	Radish	95.3	0.80	0.80	0.05
Chicory leaves	94.5	0.23	0.35	0.13	Spinach	91.4	0.07	0.05	0.05
Celery	88.0	0.16	0.22	0.02	Tomato	94.5	1.25	1.37	0.00
Cucumber	95.2	0.76	0.87	0.03	Yam	69.6	0.91	2.62	0.00

4.2.3 Physiology and nutrition

The key compound of carbohydrate metabolism, and a source of energy in animals and plants, is glucose. Heterotrophic organisms obtain energy for endergonic reactions by oxidation of glucose and other primary nutrients. Carbohydrates should account for more than 55% of energy. About 80–90% of the energy intake provided by carbohydrates should come from polysaccharides, with up to 20% coming from oligosaccharides and monosaccharides.

The ingested polysaccharides and oligosaccharides must be hydrolysed to their component monosaccharides before being absorbed. The digestion of starch starts by the action of salivary α-amylase and continues with pancreatic α-amylase in the small intestine. The hydrolysis yields α-dextrins, maltose oligomers, maltose and small amounts of glucose. Maltase and isomaltase from the lining of the small intestine (so-called brush border hydrolyases) split the products produced by amylases into glucose. Saccharose is digested in the lining of the small intestine by saccharase (sucrase) into glucose and fructose. Lactose is hydrolysed to glucose and galactose by lactase, which is also found in the intestinal lining, but is absent in most adult humans (see Section 10.3.1.1). Other carbohydrates, for example pectins, pass undigested into the large intestine and are partly hydrolysed by intestinal bacteria. Some carbohydrates, such as cellulose, are not digested at all, as humans lack the enzyme cellulase. After digestion, the small intestine (mainly duodenum) absorbs the available glucose and other monosaccharides and carries them into enterocytes (see Section 3.4.3.2.2). Only glucose and galactose are actively absorbed (by co-transport with sodium using the same hexose transporter), while fructose passes into the enterocyte via facilitated diffusion through another hexose transporter. Glucose, galactose and fructose are tranported out of the enterocyte through another hexose transporter, and then diffuse into blood capillaries within the villus.

Blood glucose concentrations (glycaemia) are controlled by three hormones: insulin, glucagon and epinephrine (adrenaline). If the concentration of glucose in the blood is too high, insulin is secreted by the pancreas and stimulates the transfer of glucose into the liver, muscles and other organs that are able to metabolise glucose by the metabolic process called **glycolysis** to give pyruvic acid and ATP. Since glycolysis releases relatively little ATP, pyruvic acid is converted into acetyl-CoA, which enters the citric acid cycle. Glucose oxidation yields simple organic compounds and the final products of oxidation are carbon dioxide and water. During strenuous muscular activity, pyruvic acid is converted into lactic acid, which is transformed during the resting period back into pyruvic acid. Pyruvic acid in turn yields glucose by a process called **gluconeogenesis**. Excess glucose is stored in the liver and muscles as polysaccharide glycogen, which is formed by the process of **glycogenesis**. Glycogen is stored in the liver and muscles until the blood glucose levels are low. Then epinephrine and glucogon hormones are secreted to stimulate the transformation of glycogen into glucose by the process called **glycogenolysis**.

The blood glucose level induced by carbohydrates in food can be measured by the glycaemic index (GI), which evaluates the biological value of dietary carbohydrates and is a measure of the effects of food carbohydrates on the blood glucose level. It is defined as the glycaemic response elicited by a 50 g portion of carbohydrate food, and expressed as a percent of that elicited by 50 g of D-glucose (GI = 100). For comparison, D-frucose has a GI value of 19, D-glucitol 9, xylitol 13 and D-mannitol 0. Rice (white) has a GI value of 81, milk (whole) 39, apples 52 and banana 83.

The main pathway of galactose metabolism is the Leloir pathway, in which, in the liver, β-D-galactose is converted into UDP-α-D-glucose (a precursor of glycogen and a building block of nucleotide metabolism) via α-D-galactose, α-D-galactose 1-phosphate and UDP-α-D-galactose under the action of mutarotase, galactokinase, galactose 1-phosphate uridyltransferase and galactose-4′-epimerase, respectively. In the liver, fructose is phosphorylated to D-fructose 1-phosphate by fructokinase. Fructose phosphate is split by aldolase B into dihydroxyacetone phosphate (identical to the dihydroxyacetone phosphate produced by glycolysis) and glyceraldehyde. Under the action of triokinase, glyceraldehyde is transformed into glyceraldehyde 3-phosphate. The resulting triose phosphates can enter the gluconeogenic pathway, and can be used for glucose or glycogen synthesis, or be further catabolised.

Exogenous polyols are absorbed slowly and metabolised mainly by the hepatic enzymes. D-Glucitol (sorbitol) and xylitol are metabolised completely after oxidation. Glucitol is oxidised to fructose by glucitol dehydrogenase. Xylitol is oxidised to D-xylulose by xylitol dehydrogenase and xylulose is phosphorylated by xylulose kinase to D-xylulose 5-phosphate, which is an intermediate of the pentose phosphate pathway. D-Mannitol is poorly utilised because of the low affinity for glucitol dehydrogenase. Exogenous erythritol is very poorly metabolised, being almost completely excreted in the urine.

4.2.4 Use

Glucose and glucose syrup are raw material for the production of D-glucitol, D-mannitol, D-fructose, D-gluconic acid and its lactones, various fat and sugar substitutes, such as an indigestible synthetic polymer of glucose called polydextrose having predominantly α-(1→6) bonds. Polydextrose is obtained by fusion of glucose with glucitol and citric acid. Glucose is still the most important raw material for ethanol production by fermentation.

4.3 Derivatives of monosaccharides

4.3.1 Sugar alcohols

Reduction of carbonyl groups of aldoses and ketoses gives rise to acyclic polyhydroxy derivatives of hydrocarbons called sugar alcohols, polyols or alditols (formerly also known as glycitols). Reduction of aldoses produces only one sugar alcohol, reduction of ketoses creates a new asymmetric carbon and the products are two diastereoisomeric polyols. Polyols formed by reduction of some sugars have a plane of symmetry that bisects a molecule into halves that are mirror images of each other and the polyols are therefore achiral meso forms. Through the hydrophilic hydroxyl groups, sugar alcohols are involved in hydration of macromolecules.

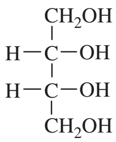
They also act in the prevention of various stress factors and lipid peroxidation in plant cells.

Other saccharides (sugar alcohols) also include cyclitols (alicyclic polyols), because they have similar properties, although they are not derived from sugars by simple reduction. Cyclitols are cycloalkanes, in which at least three ring carbon atoms are substituted (each of them only once) by hydroxyl or alkoxyl groups.

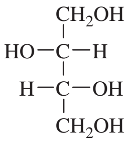
4.3.1.1 Structure and nomenclature

4.3.1.1.1 Alditols

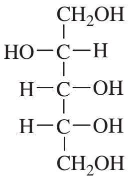
The simplest alditol, which is derived from D-glyceraldehyde, L-glyceraldehyde or 1,3-dihydroxyacetone, is glycerol (propane-1,2,3-triol).[1] The name of the optically active higher alditol is derived from the name of sugar from which the alditol is derived and to which the suffix -itol is connected (e.g. erythritol and threitol). From the four tetroses (aldoses of D- and L-series), only three tetritols can be derived by reduction. Erythritol (**4-47**), which is a meso form, arises from the D- or L-erythrose; D-threitol (**4-48**) is formed from D-threose and L-threitol from L-threose. Aldopentoses of D- and L-series (total of eight aldoses) provide four pentitols. D-Arabinitol (**4-49**) is formed from D-arabinose or D-lyxose, L-arabinitol from L-arabinose or L-lyxose, ribitol (**4-50**) from D- or L-ribose and xylitol (**4-51**) from D- or L-xylose. The reduction of the 16 aldohexoses of D- and L-series yields ten alditols: allitol (**4-52**), D-altritol (**4-53**), L-altritol, D-glucitol (**4-54**), formerly sometimes incorrectly called L-gulitol or more often D-sorbitol (by the Latin name *Sorbus* for rowan or mountain ash trees and shrubs in which glucitol was firstly identified in the fruits), L-glucitol, D-mannitol (**4-55**), L-mannitol, D-iditol (**4-56**), L-iditol and galactitol (**4-57**), formerly called dulcitol. For example, D-glucitol is also formed by reduction of D-glucose and L-gulose.



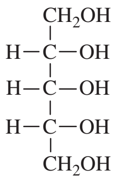
4-47, erythritol



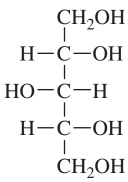
4-48, D-threitol



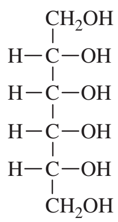
4-49, D-arabinitol



4-50, ribitol

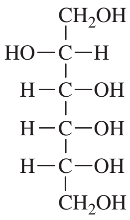


4-51, xylitol

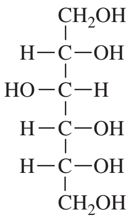


4-52, allitol

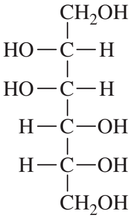
[1]Reduction of glycolaldehyde or glyoxal yields ethylene glycol (ethane-1,2-diol) and the reduction of formaldehyde gives methanol. However, these compounds are not ranked among the alditols.



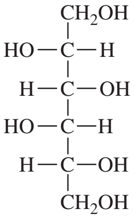
4-53, D-altritol



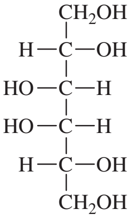
4-54, D-glucitol



4-55, D-mannitol



4-56, D-iditol



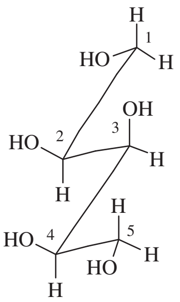
4-57, galactitol

The systematic name of D-glucitol is (2*R*,3*S*,4*S*,5*S*)-hexane-1,2,3,4,5,6-hexol, D-mannitol is (2*R*,3*R*,4*R*,5*R*)-hexane-1,2,3,4,5,6-hexol and galactitol is (2*R*,3*S*,4*R*,5*S*)-hexane-1,2,3,4,5,6-hexol.

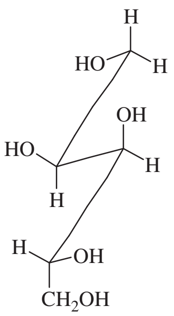
The reduction of ketoses yields both C-2 isomers. For example, the reduction of D-*glycero*-tetrulose creates erythritol and D-threitol. Reduction of D-fructose yields D-glucitol and D-mannitol.

Conformation

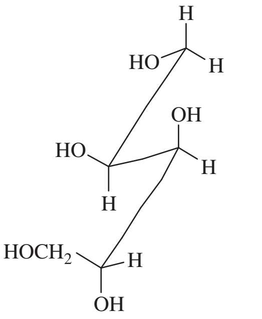
Alditols in solutions most frequently occupy the zigzag conformation with carbon atoms in one plane. The planar conformer of xylitol is shown in stereoformula **4-58** (using the Newman projection, diagrammatic formula). As in the cyclic forms of aldoses and ketoses, non-bonding syn-axial interactions also exist in planar zigzag conformers of alditols, which are the most important destabilising factor determining the relative abundance of different conformers. As a result of interactions of hydroxyl groups on C-2 and C-4 of xylitol, displaying the internal stress, the functional groups rotate around the bonds C3–C4 and the most stable conformer is **4-59** and the least stable conformer is **4-60** with the hydroxyl group at C-2 interacting with bulky hydroxymethyl group at C-4. The prevailing conformer is therefore the anticlinal (also called gauche or bent) conformer.



4-58, planar zigzag conformer of xylitol

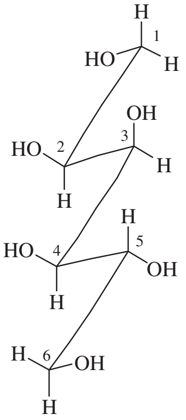


4-59, thermodynamically most stable conformer of xylitol

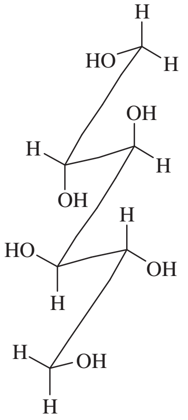


4-60, thermodynamically least stable conformer of xylitol

The syn-axial interactions also occur between the hydroxyl groups at C-2 and C-4 of ribitol, but not with D-arabinitol. Generally speaking, alditols in the configuration *xylo* largely exist as conformers of the type shown in **4-59**, and alditols in the configuration *arabino* primarily exist as planar zigzag conformers of the type shown in **4-58**. Analogously, the *syn*-axial interactions in the zigzag conformation also exist in hexitols allitol, altritol, D-glucitol (**4-61**) and D-iditol, but not with D-mannitol (**4-62**) and galactitol. Only hexitol in the *galacto* and *manno* configuration therefore occurs in the planar zigzag conformations in solutions and the predominant conformation of other hexitols is gauche with other non-planar conformations.



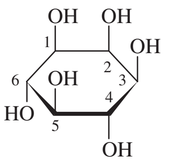
4-61, conformation of D-glucitol



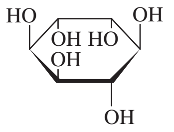
4-62, conformation of D-mannitol

4.3.1.1.2 Cyclitols

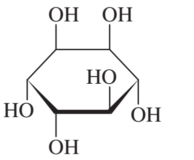
An important group of polyols are cycloalkanes, with three to six hydroxy groups bound to carbon atoms of the ring. The most important cyclitols are polyhydroxyalcohols, formally derived from cyclohexane, which are called cyclohexitols. In addition to hexahydroxy derivatives, penta-, tetra- and trihydroxycyclohexanes and their derivatives are commonly found in plants. The most important cyclohexitols are hexahydroxycyclohexanes or cyclohexane-1,2,3,4,5,6-hexols, known under the generic name **inositols** (formerly called **cycloses**). Altogether there are eight stereoisomers, which are distinguished by affixes or by numerical prefixes (locants) indicating the individual atoms of the ring (**4-63** to **4-71**). As a result of the symmetry of the molecules, seven inositols are achiral substances, optically inactive meso forms, *chiro*-inositol is the only cyclohexitol that occurs as the (+)-D- - or (−)-L-enantiomer. Formerly, this substance has been called inositol without the affix. The affix *chiro* was chosen so as to underline that *chiro*-inositol is the only optically active inositol.



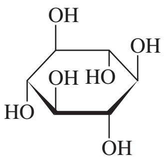
4-63, *myo*-inositol



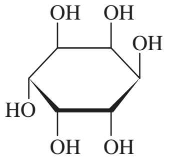
4-64, 1D-(+)-*chiro*-inositol



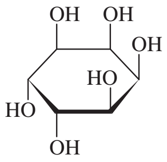
4-65, 1L-(−)-*chiro*-inositol



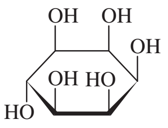
4-66, *scyllo*-inositol



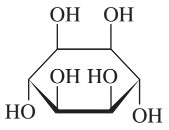
4-67, *neo*-inositol



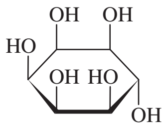
4-68, *allo*-inositol



4-69, *epi*-inositol



4-70, *muco*-inositol



4-71, *cis*-inositol

The most important compound is *myo*-inositol, (1*Z*,2*Z*,3*Z*,5*Z*,4*E*,6*E*)-cyclohexane-1,2,3,4,5,6-hexol, formerly also called *meso*-inositol (or i-inositol, phaseomannitol, nucitol, bios I, mouse antialopaecia factor or vitamin B_m). The affix *myo* was preferred to the affix *meso* as it defines a certain configuration of hydroxyl groups above and below the plane of the ring (1,2,3,5/4,6-), while the second affix has a general meaning and is used to label achiral, optically inactive compounds with the same number of identically bound enantiomeric groups.

Conformation

Analogously to pyranoses, cyclohexitols and derived carboxylic acids occur in sterically stable chair conformations. The lowest energy has *scyllo*-inositol with all substituents in equatorial positions.

4.3.1.2 Occurrence

4.3.1.2.1 Alditols

Alditols occurring in foods as natural components are the result of biochemical reactions, but they can also arise as products of Cannizzaro reaction or other non-enzymatic browning reactions. Many synthetic alditols are used as food additives (sweeteners; see Section 11.3.2.2).

The simplest alditol, glycerol, is a component of food lipids and also occurs as a byproduct of fermentation in addition to

(2*R*,3*R*)-butane-2,3-diol, also known as (−)-D-butane-2,3-diol. It is accompanied by a small amount of (2*S*,3*S*)-butane-2,3-diol, also known as (+)-L-butane-2,3-diol, and *meso*-2,3-butane-2,3-diol. The glycerol and (2*R*,3*R*)-butane-2,3-diol contents can be used as an indicator of wine quality. Erythritol is found in significant quantities in some algae, and small amounts are present in wine musts (juices) and hence, along with glycerol and other alditols, is found in wines (Table 4.7). The content of alditols in wines is higher or the same as in the musts and depends on the type of yeast and other factors. Ribitol is a constituent of riboflavin; D-arabinitol and xylitol are found fairly frequently in fruits, vegetables and mushrooms. Mushrooms (such as the common white button mushroom, *Agaricus bisporus*) contain D-arabinitol at levels of about 3.5 g/kg dry matter and xylitol at about 1.3 g/kg dry matter.

Widely spread alditols in foods are hexitols, particularly D-mannitol, which is the most common hexitol. Other common hexitols are D-glucitol and galactitol. The accumulation of D-mannitol in plants increases their resistance to the high salinity of soil. Fruits and vegetables usually contain very low amounts of mannitol (up to 0.01–0.02%); a relatively high mannitol content is found in celery (0.48%). Its content in green (unroasted) coffee is around 0.05%, whilst in soluble coffee the mannitol content ranges from 0.20 to 1.03%, and waste from the production of soluble coffee contains 1.61–2.03% mannitol. These wastes are therefore used as a raw material for isolating mannitol. Higher amounts of mannitol in wines (1–30 g/l) may be caused by attack of the *Botrytis cinerea* fungus invading the grapes, or by activities of bacteria *Bacterium mannitopoeum*. The fermentation type is called mannitol fermentation or slimy fermentation of sugars. Higher fungi can have a very high mannitol content. The level of mannitol in fungi *Tricholoma portentosum*, commonly known as the charbonnier, is 1.0%, in Saffron milk cap (*Lactarius deliciosus*) 13.7%, in horse mushroom (*Agaricus arvensis*) 6.5% and in the cultivated common mushroom (*Agaricus bisporus*) 15.7% in dry matter of young fruiting bodies, but 26% in dry matter of mature fruiting bodies. The mannitol content in brine of canned common mushrooms is 2.5–10.5 g/l.

The D-glucitol contents in some common fruits and vegetables are given in Table. 4.8. For example, rowan berries, together with the main hexitol D-glucitol, also contain D-mannitol and D-iditol. Prunes have a higher amount of glucitol, and for this reason they are used in various laxative preparations.

A higher concentration of galactitol is found in fungi and fermented milk products. Mushrooms have a galactitol content of about 0.5 g/kg dry matter, while about 9 g/kg dry matter can be found in yoghurts.

Table 4.7 Main alditols in wines.

Alditol	Content (mg/l)	Alditol	Content (mg/l)
Glycerol	4 000–10 000	Xylitol	4–11
Erythritol	35–292	D-Glucitol	9–277
D-Arabinitol	10–577	D-Mannitol	6–152

Table 4.8 D-Glucitol in fruits and vegetables.

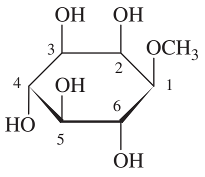
Fruits and vegetables	Content (% of edible portion)	Fruits and vegetables	Content (% of edible portion)
Apples	0.2–0.8	Apricots	0.05–0.46
Pears	1.2–2.8	Rowan berries	3.4–5.3
Plums	0.6–13.9	Grapes	0.01
Cherries	0.1	Cucumbers	0.01
Peaches	0.03–0.48	Tomatoes	0.01

4.3.1.2.2 Cyclitols

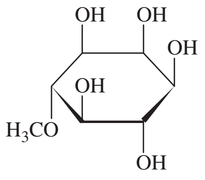
myo-Inositol is found as a free compound in fruits and vegetables, where its content ranges from 0.01 to 0.05%, 0.02–0.30% of *myo*-inositol is present in legumes, cereals and nuts, particularly in bound forms. In legumes, cereals and nuts, *myo*-inositol is mainly present as *myo*-inositol-1,2,3,4,5,6-hexakisdihydrogenphosphoric acid, known as phytic acid, occurring in the form of a mixed K^+, Mg^{2+}, Ca^{2+}, Fe^{2+} and Zn^{2+} salt called phytin (see Section 6.3.4.2.2). Phytin is the main bound form of phosphorus used during seed germination. It also occurs in other parts of plants, such as pollen, roots and tubers. For example, wheat contains about 10 g/kg of phytin. Approximately 70–80% of phytin in dough is hydrolysed by the yeast enzyme phytase to *myo*-inositol. *myo*-Inositol also commonly occurs in the form of phospholipids (phosphatides), known as phosphoinositols. In legumes, buckwheat and other crops, *myo*-inositol is accompanied by a minority cyclitol 1D-*chiro*-inositol and in grape musts by *scyllo*-inositol.

myo-Inositol is a precursor for the biosynthesis of other cyclitols, *O*-methylinositols and other derivatives. Methylation of *myo*-inositol and isomeric inositols (*scyllo*-, *chiro*-, *muco*- and *neo*-inositol) provides *O*-methylinositols bornesitol (1D-1-*O*-methyl-*myo*-inositol; **4-72**), ononitol (1D-4-*O*-methyl-*myo*-inositol; **4-73**), sequoitol (1D-5-*O*-methyl-*myo*-inositol; **4-74**), pinitol (1D-4-*O*-methyl-*chiro*-inositol; **4-75**), quebrachitol (1L-2-*O*-methyl-*chiro*-inositol; **4-76**) and others that are involved in a plant's adaptability to various forms of stress, used as seed storage substances and building blocks for biosynthesis of glycosides. D-Sequoitol occurs as a minor component in beans and in many other plants. A common methyl ether of legumes, derived from D-*chiro*-inositol, is D-pinitol.

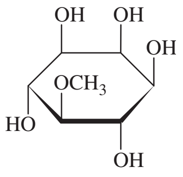
Cyclitols (hexahydroxycyclohexanes) and their ethers also occur in a variety of α-D-galactosides, which are called



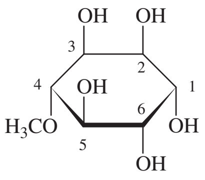
4-72, D-bornesitol



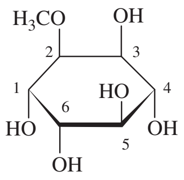
4-73, D-ononitol



4-74, D-sequoitol

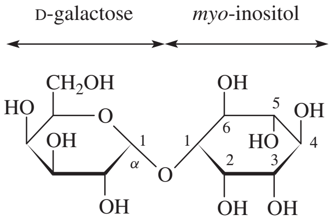


4-75, D-pinitol



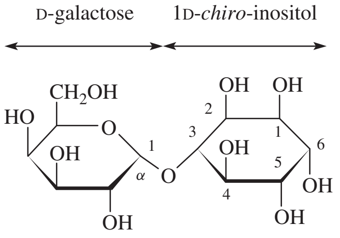
4-76, L-quebrachitol

pseudooligosaccharides. Conjugation of *myo*-inositol and galactose yields galactinol, known by its systematic name as 3-*O*-α-D-galactopyranosyl-D-*myo*-inositol, or also as 1-*O*-α-D-galactopyranosyl-L-*myo*-inositol (**4-77**), the most famous and the first representative of pseudooligosaccharides to be identified. It occurs in all plants containing triose raffinose and higher α-galactooligosaccharides (stachyose, verbascose and ajugose) and therefore occurs in all legumes, as it is employed as the starting compound for the biosynthesis of galactooligosaccharides. Also present are higher α-D-galactopyranosyl homologues of galactinol. The highest concentration of these compounds is found in germs of mature seeds.

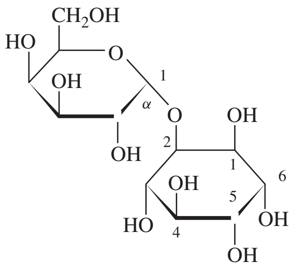


4-77, galactinol

The reaction of 1D-*chiro*-inositol with D-galactose provides fagopyritols (**4-78** to **4-81**), which are present in legumes. Fagopyritols occur at higher concentrations in the seeds and especially in the germ of buckwheat seeds (*Fagopyrum esculentum*, Fabaceae). Fagopyritols of the A series are 3-*O*-α-D-galactopyranosyl-1D-*chiro*-inositols, fagopyritols of the B series are 2-*O*-α-D-galactopyranosyl-1D-*chiro*-inositols.

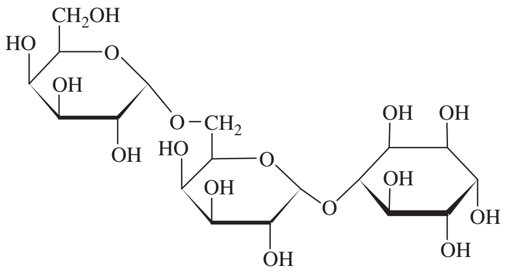


4-78, fagopyritol A1

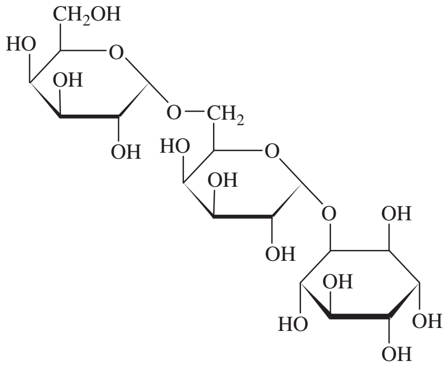


4-79, fagopyritol B1

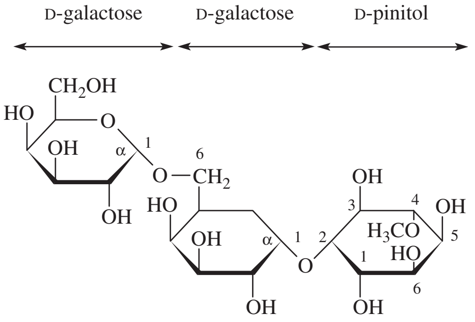
Galactinol in soybeans and other legumes is accompanied by α-D-galactosides of pinitol, known as galactopinitols. A common compound is α-D-digalactopyranoside of pinitol called ciceritol, or by the systematic name *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→2)-4-*O*-methyl-1D-*chiro*-inositol (**4-82**). Contents of major cyclitols and their galactosides occurring in legumes are given in Table 4.9.



4-80, fagopyritol A2



4-81, fagopyritol B2



4-82, ciceritol

4.3.1.3 Physiology and nutrition

4.3.1.3.1 Alditols

The most important hexitols are D-glucitol and D-mannitol. The most important pentitol is xylitol. The relative sweetness of both hexitols (in 10% solutions) and xylitol, compared with saccharose, is about 60 and 100%, respectively. All three polyols have very little effect on blood glucose level and are therefore used as sweeteners for diabetics. However, their content must be included in the total energy intake. They exhibit mild laxative effects (such as prunes, which contain higher amounts of glucitol than other fruits).

Xylitol exposure has an inhibiting effect on the growth of cariogenic bacteria and this may create a permanent change in their oral population. Under discussion are the anticariogenic effects of xylitol. The sweetness and pleasant cooling effect of xylitol sweetened products in the mouth (such as mints and chewing gums) create

Table 4.9 Important cyclitols and their galactosides in legumes (% of dry matter).

Legume	Latin name	*Myo*-inositol	D-Pinitol	Galactinol	Galactopinitols[a]	Ciceritol
Beans	*Phaseolus vulgaris*	0.02–0.06	0.08–0.20	0.04–0.05	0.00–0.04	traces
Peas	*Pisum sativum*	0.10–0.17	0.05	0.07	0.00	0.00
Lens	*Lens culinaris*	0.07–0.11	1.11–0.40	0.10–0.12	0.36–0.39	1.60
Soybeans	*Glycine max*	0.03–0.10	0.20–0.90	0.00	0.35–0.70	0.08
Chickpeas	*Cicer arietinum*	0.10–0.30	0.40–0.45	0.08–0.20	0.50–0.80	2.80

[a] Sum of 2-*O*-α-D-galactopyranosyl-1D-*chiro*-inositol (fagopyritol B1) and D-pinitol derivatives, 2-*O*-α-D-galactopyranosyl-4-*O*-methyl-1D-*chiro*-inositol and 5-*O*-α-D-galactopyranosyl-4-*O*-methyl-1D-*chiro*-inositol.

an increase in salivary flow. The cooling effect of xylitol is produced in its contact with saliva due to its negative heat of solution (−149 kJ/kg, which corresponds to −27 kJ/mol). Other polyols have a much lower negative enthalpy of dissolution. D-Glucitol and D-mannitol are only weakly cariogenic.

In individuals with galactosaemia (type 1), a rare disorder that affects galactose metabolism (see Section 4.2.3), galactose accumulates in the body tissues together with galactitol, which is an alternative product of degradation. Galactosaemia, which is especially dangerous in children, is caused by the lack of the enzyme galactose-1-phosphate uridyltransferase. Galactitol is toxic to the liver (which may lead to failure), brain (mental retardation) and kidney and to the eye lens (cataract).

4.3.1.3.2 Cyclitols

In nature, the most common cyclitol is *myo*-inositol. It is the key compound in the metabolism of microorganisms, plants and animals, therefore it was formerly considered one of the vitamins (see Section 5.15), but the *myo*-inositol derivative phytin (see Section 10.2.3.1) is classified as an antinutritional food constituent.

4.3.1.4 Use

Polyols xylitol, D-glucitol and D-mannitol have found extensive use primarily as sweeteners in foods for diabetics. They are used in many bakery and confectionery products (mainly xylitol and D-glucitol) to reduce water activity, as substances suppressing crystallisation of saccharose and as humectants improving hydration of dry goods.

Alditols are mainly produced from aldoses or ketoses by hydrogenation or reduction with amalgams, complex hydrides or by electrolytic reduction. Xylitol is prepared by reduction of D-xylose obtained by hydrolysis of natural xylems (hemicelluloses), D-glucitol is prepared from D-glucose and D-mannitol via hydrogenation of D-fructose obtained from either starch or invert sugar.

Also some other polyols may find some application in nutrition (as sweet substances). Examples of these polyols include L-arabinitol (sweetening power of about 100%) obtained by hydrogenation of L-arabinose, galactitol (40%) obtained from D-galactose and polyols derived from disaccharides.

4.3.2 Sugar acids

Sugar acids are saccharides with carboxyl group(s) that commonly occur in foods as free substances and components of many oligosaccharides, polysaccharides, heteroglycosides and other food constituents. Frequently these acids are formally derived from monosaccharides by oxidation of aldehyde groups or primary alcohol groups. Sugar acids are usually produced by enzymatic reactions, but some sugar acids derived from hexoses (such as saccharinic acids) and lower sugars are formed by chemical reactions during storage and processing of foods. Of particular importance in this respect is the Maillard reaction.

Some carboxylic acids, hydroxy acids and oxoacids produced by glycolysis, arising in the citric acid cycle, in the glyoxylate cycle, or their modifications and can thus also be considered sugar derivatives. An example of C_6 substances is L-ascorbic acid and other forms of vitamin C, 2-oxoglutaric acid is a C_5 substance, examples of C_4 substances are tartaric and malic acids, C_3 substances are glyceric, lactic and pyruvic acids and a C_2 substance is glyoxylic acid. Sugar acids also include carboxylic acids derived from alicyclic sugar alcohols (cyclitols) (**8-71**).

4.3.2.1 Structure and nomenclature

Oxidation of aldehyde groups of aldoses yields aldonic (generally glyconic) acids.[2] Free acids readily (especially in acidic solutions) lactonise to give relatively stable five-membered γ-lactones or less stable six-membered δ-lactones. Nomenclature of aldonic acids and their lactones is based on the substitution of the suffix -ose in the aldose name with the suffix -onic acid or -onolactone, respectively. For example, D-glucose gives rise to D-gluconic acid and its dehydration yields the corresponding gluconolactones (Figure 4.3). Another common type of sugar acids are uronic (alduronic) acids, which are derived by oxidation of primary hydroxyl groups. The name of an uronic acid is formed by replacing the suffix -ose with

[2] Under more drastic conditions (e.g. by oxidation with nitric acid), the terminal hydroxymethyl group of aldonic acids is oxidised to a carboxyl group, which produces dicarboxylic acids known as aldaric acids that are usually called glycaric acids. D-Glucose yields D-glucaric acid (also known as saccharic acid), from D-galactose arises achiral galactaric (mucic) acid.

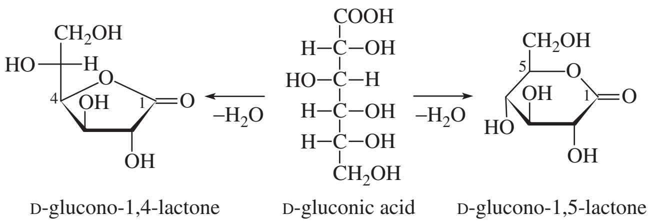


Figure 4.3 Formation of D-gluconic acid lactones.

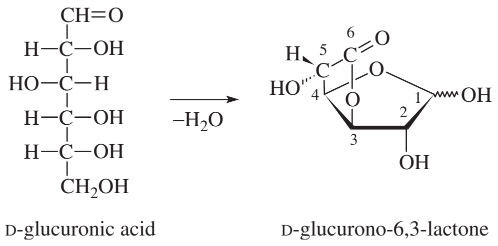


Figure 4.4 Formation of D-glucuronic acid lactones.

the suffix -uronic acid. Uronic acids easily produce 6,3-lactones of furanose or pyranose forms. D-Glucose thus forms D-glucuronic acid (occurring as pyranose) and its lactone (Figure 4.4), D-galactose yields D-galacturonic acid and D-mannuronic acid is derived from D-mannose.

The most important alicyclic acid derived from tetrahydroxycyclohexane is (3*R*,5*R*)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid known as (−)-L-quinic acid. The trihydroxycyclohexene derivative is (3*R*,4*S*,5*R*)-3,4,5-trihydroxycyclohexene-1-carboxylic acid known as (−)-L-shikimic acid (see **8-71**).

4.3.2.2 Occurrence

Gluconic and mannonic acids (as well as glycolic, glyceric, lactic and pyruvic acids) are present in relatively large amounts as natural components in many plant materials, as well as in those foods in which non-enzymatic browning reactions take place. Table 4.10 illustrates the composition and content of aldonic, deoxyaldonic and some other acids in chicory root and malt.

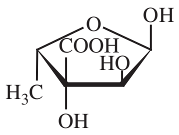
Alduronic acids, especially D-glucuronic, D-galacturonic, D-mannuronic and L-guluronic acids occur chiefly as the building blocks of some polysaccharides. Constituents of rhamnogalacturonan chains in pectin (see Section 4.5.6.6) are unusual acids such as aceric acid (3-*C*-carboxy-5-deoxy-β-L-xylofuranose, β-L-Ace*f*A, **4-83**), deoxyheptonic acid (3-deoxy-β-D-*lyxo*-hept-2-ulopyranaric acid, β-D-Dha*p*, **4-84**) and ketodeoxyoctonic acid (3-deoxy-β-D-*manno*-oct-2-ulopyranosonic acid, β-D-Kdo*p*, 4-85), which is also a component of lipopolysaccharides of gram negative bacteria.

Alicyclic acids, quinic acid and shikimic acid (intermediates in the biosynthesis of phenylalanine, tyrosine and tryptophan, numerous flavourings, natural colourings and other compounds), are common compounds in foods. They occur as free compounds and also in the form of various derivatives, for example in depsides such as chlorogenic acids (**8-82**).

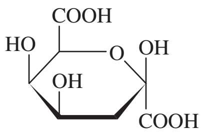
Table 4.10 Sugar and related carboxylic acids in chicory root and malt (g/kg of dry matter).

Carboxylic acids	Chicory root			Malt	
	Fresh	Dried	Roasted[a]	Dried	Roasted[a]
Aldonic					
Glycolic	0.46	0.15	0.94	0.03	0.10
Glyceric	0.15	0.05	0.24	0.04	0.11
Erythronic	0.00	0.03	0.09	0.00	0.04
Threonic	0.00	0.01	0.07	0.01	0.07
Ribonic	0.00	0.04	0.07	0.03	0.08
Arabinonic	0.00	traces	0.13	traces	0.02
Gluconic	0.46	0.03	0.08	0.58	0.30
Mannonic	0.30	0.03	0.05	0.20	0.15
Deoxyaldonic					
Lactic (2-hydroxy-propanoic)	0.45	0.45	0.80	0.19	0.33
3-Hydroxy-propanic	0.00	0.12	0.31	0.08	0.21
2.4-Dihydroxy-butanoic	0.00	0.00	0.06	0.04	0.08
3,4-Dihydroxy-butanoic	0.00	0.00	0.15	0.05	0.20
α-D-Glucometa-saccharinic (3-deoxy-D-*ribo*-hexonic)	0.00	0.02	0.32	traces	0.07
β-D-Glucometa-saccharinic (3-deoxy-D-*arabino*-hexonic)	0.00	0.04	0.36	0.02	0.06
α-D-Isosaccharinic	0.00	traces	traces	0.00	traces
α-D-Glucosaccharinic	0.00	0.00	traces	0.00	0.02
Various					
Pyruvic (2-oxopropanoic)	0.30	0.11	0.43	0.49	1.06
Laevulinic (4-oxopentanoic)	0.00	0.00	traces	0.00	0.03
Furan-2-carboxylic (pyromucic, furoic)	0.00	0.03	0.07	0.00	0.03
5-Hydroxy-methylfuran-2-carboxylic	0.00	0.00	0.15	0.00	0.06

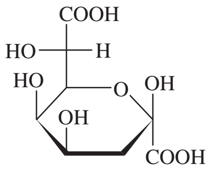
[a]At 170 °C.



4-83, aceric acid



4-84, deoxyheptonic acid



4-85, ketodeoxyoctonic acid

4.3.2.3 Physiology and nutrition

6,3-Glucuronolactone is used in the diet of athletes as a popular supplement for body building. It was found that it accelerates formation and decelerates degradation of muscle glycogen. Glycosides of γ-lactone of D-glucuronic acid, called D-glucuronides, act in the body in detoxification reactions.

4.3.2.4 Use

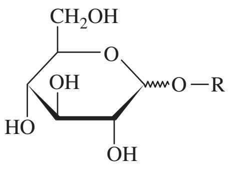
δ-D-Gluconolactone is added at levels of around 0.1% to some smoked meat products, such as fermented sausages and salami. Gradually hydrolysed lactone produces free gluconic acid that inhibits the growth of undesirable putrid microflora, especially at the beginning of product ripening together with lactic and acetic acids produced by fermentation of gluconic acid by some bacteria of the genus *Lactobacillus*.

In dermatology, gluconolactone is capable of chelating metals and may also function by scavenging free radicals, thereby protecting the skin from some of the damaging effects of UV radiation.

4.3.3 Other sugar derivatives

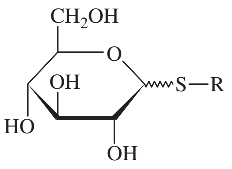
4.3.3.1 Glycosides

The reaction of the hemiacetal hydroxyl group of sugars with hydroxy compounds yields sugar glycosides also known as *O*-glycosides. For example, glycosides derived from glucose are glucosides and mannose gives rise to mannosides (**4-86**). The non-sugar part of the glycoside molecule is generally termed **aglycone** (formerly also referred to as genine). Aglycones are mostly phenols, alicyclic triterpenic alcohols and other steroids (see Section 3.7.4), but can also be other hydroxy compounds. Glycoside is then called a **heteroglycoside**. If the reacting hydroxy compound is another sugar, the resulting product is a **homoglycoside**. Homoglycosides are oligosaccharides and polysaccharides, which are common food components. They form mainly in reactions catalysed by enzymes, but also in other reactions, for example by reversion of monosaccharides.



4-86, *O*-glycoside derived from D-glucose

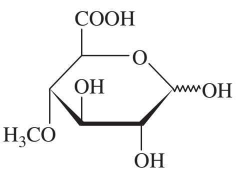
Other natural food components, such as *S*-glycosides, known as thioglycosides, can also be found (**4-87**). Examples of these compounds are glucosinolates (see Section 10.3.2.4). Nitrogen analogues of glycosides are *N*-glycosides (glycosylamines). They occur as natural food components and are also formed in the Maillard reaction. Glycosides similarly include *C*-glycosides, which are actually derivatives of anhydroalditols. Formally, *C*-glycosides resemble glycosides, but are not hydrolysed with acids to form the parent aglycone and sugar. Examples of these glycosides include some natural dyes, such as carminic acid, carthamin, mangiferin and aloin. Some *C*-glycosides also arise as products of the Maillard reaction.



4-87, *S*-glycoside derived from D-glucose

4.3.3.2 Ethers

Ethers of sugars occur in foods as minor structural units of some polysaccharides. For example, 4-*O*-methyl-D-glucuronic acid (**4-88**) is a component of hemicelluloses (arabinoxylans) and other saccharides, 4-*O*-methyl-D-galacturonic acid, 2-*O*-methyl-D-xylose and 2-*O*-methyl-L-fucose are components of pectin. Starch and cellulose ethers are used as food hydrocolloids.

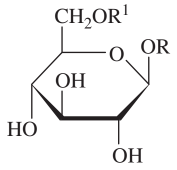


4-88, 4-*O*-methyl-D-glucuronic acid

4.3.3.3 Esters

Esters of saccharides are natural components of virtually all foods. The most common sugar esters are phosphoric acid esters (phosphates), which serve as key intermediates in metabolism by animals, plants and microorganisms. Examples are phosphates of D-glucose (D-glucose 1-phosphate and D-glucose 6-phosphate, **4-89**), but many other sugar phosphates also take part in the metabolic

processes. Phosphate esters also include metabolically active sugar nucleotides, such as adenosine diphospho-D-glucose (ADP-D-Glc), which has a role in the biosynthesis of starch. Of particular importance is D-ribose 5-phosphate, a component of pyrimidine and purine nucleotides in ribonucleic acids (RNA), free nucleotides (such as ATP) and also in many important cofactors of oxidoreductases and transferases, such as NADH and $FADH_2$.

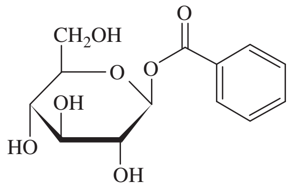


4-89, β-D-glucopyranose 1-phosphate, $R = PO_3H_2$, $R^1 = H$
β-D-glucopyranose 6- phosphate, $R = H$, $R^1 = PO_3H_2$

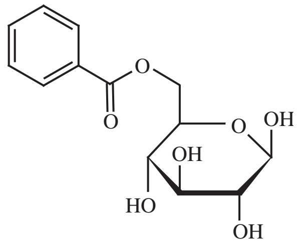
Esters with sulfuric acid often occur as components of some polysaccharides. Sugar sulfates are particularly common components in proteoglycans (mucoproteins) of animal tissues as building blocks of mucopolysaccharides. They are also components of seaweed polysaccharides agar and carrageenan.

Common sugar esters are esters with acetic acid. In animal glycoproteins (such as milk, eggs and blood serum), proteoglycans (mucoproteins), connective tissue polysaccharides and chitin are present, such as *N*-acetates of D-glucosamine, D-galactosamine and *N*-acetylneuraminic acid. Acetates of sugars are often found in various glycosides (e.g. in saponins; see Section 10.3.2.2).

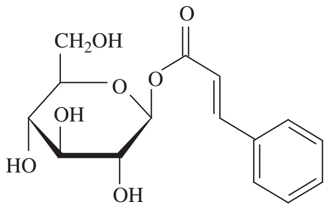
Some very widespread compounds are the esters of D-glucose with aromatic acids. A common metabolite is 1-*O*-benzoyl-β-D-glucopyranose (**4-90**), which occurs, for example, in cranberries (*Vaccinium vitis-idaea*, Ericaceae) together with 6-*O*-benzoyl-β-D-glucopyranose (**4-91**), known as vaccinin. (*E*)-1-*O*-Cinnamoyl-β-D-glucopyranose (**4-92**) has been found in strawberries (*Fragaria vesca*, Rosaceae) and cashew apples (*Anacardium occidentale*, Anacardiaceae). Hydrolysable tannins are esters of glucose with gallic acid and its derivatives (see **8-72**).



4-90, 1-*O*-benzoyl-β-D-glucopyranose



4-91, vaccinin

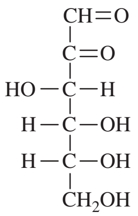


4-92, (*E*)-1-*O*-cinnamoyl-β-D-glucopyranose

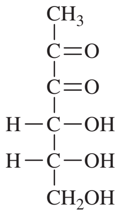
Synthetic esters of some sugars and sugar alcohols with fatty acids are used as additives, primarily as emulsifiers.

4.3.3.4 Ketoaldoses and diketoses

Monosaccharides containing both the aldehyde group and the keto group in the molecule are called **ketoaldoses** (aldoketoses or aldosuloses). If monosaccharides contain two keto groups, they are called **diketoses**. The names of ketoaldoses are formed by replacing the suffix -e in the name of aldose by the suffix –ulose, and using a suitable prefix (e.g. pento- or hexo-). The hexosulose **4-93** is D-*arabino*-hexos-2-ulose (formerly also referrd to as D-glucosulose or D-glucosone). The hexosulose arising from D-galactose during milk processing is D-*lyxo*-hexos-2-ulose (D-galactosulose or D-galactosone). The names of diketose are formed by replacing the suffix -se by the suffix -diulose. Sugars containing three keto groups are triuloses. The diketose in formula **4-94** is hexodiulose, which has the systematic name 1-deoxy-D-*erythro*-hexo-2,3-diulose. Deoxyglycosuloses are the key Maillard reaction products.



4-93, D-*arabino*-hexos-2-ulose



4-94, 1-deoxy-D-*erythro*-hexo-2,3-diulose

Hydroxymethylglyoxal can also be considered to be a ketoaldose, and methylglyoxal is deoxyketoaldose and biacetyl is dideoxydiketose. All these compounds are formed as degradation products of sugars. Other products of sugar fragmentation include deoxyaldose 2-hydroxypropanal (lactic aldehyde), deoxyketose hydroxyacetone (acetol or pyruvic acid aldehyde) and dideoxyketose acetoin (3-hydroxybutan-2-one).

4.3.3.5 Anhydrosugars

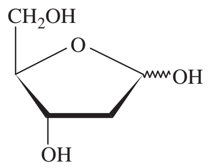
Sugar anhydrides or **anhydrosugars** were previously generally termed **glycosans**. Anhydrosugars formed from glucose were known as glucosans, anhydrosugars derived from mannose were mannosans and anhydrosugars arising from fructose were fructosans. Anhydrosugars are formed by intramolecular condensation of the hemiacetal hydroxyl group and some other hydroxyl groups

in the sugar molecule when it is heated in acidic solutions or in solid form. The anhydrosugar derived from D-glucose, 1,6-anhydro-β-D-glucopyranose (also known as β-glucosan or laevoglucosan), and other anhydrosugars are therefore normal constituents of caramel.

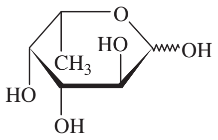
Polysaccharide agar is composed of 3,6-anhydro-α-L-galactopyranose and polysaccharide carrageenan of 3,6-anhydro-α-D-galactopyranose.

4.3.3.6 Deoxysugars

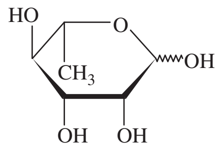
Deoxysugars are sugar derivatives in which one or more hydroxyl groups (but not the semiacetal hydroxyl) are replaced by a hydrogen atom. In foods, deoxysugars are bound in glycosides, glycoproteins or bacterial lipids. Important deoxysugars occurring in foods are 2-deoxy-D-ribose (formerly known as thyminose or 2-deoxy-D-*erythro*-pentofuranose, **4-95**), which is a constituent of deoxyribonucleic acids, and L-fucose (6-deoxy-L-galactopyranose, **4-96**), which occurs as a constituent of milk oligosaccharides. Deoxysugars L-rhamnose (6-deoxy-L-mannopyranose, **4-97**) and D-chinovose (6-deoxy-D-glucopyranose, **4-98**) are constituents of many glycosides. Various deoxysugars, especially deoxyglycosuloses, arise as intermediate products during degradation of monosaccharides in acidic media and as the Maillard reaction products.



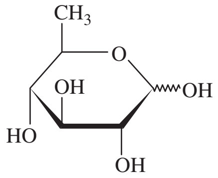
4-95, 2-deoxy-D-ribose



4-96, L-fucose



4-97, L-rhamnose

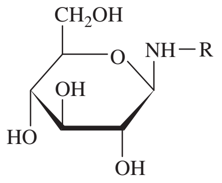


4-98, D-chinovose

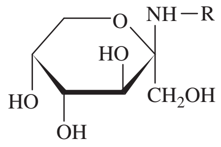
The properties of deoxysugars are similar to those of sugars, but their glycosides, especially glycosides of 2-deoxysugars, are hydrolysed faster.

4.3.3.7 Amino derivatives

Hydroxyl groups of saccharides can be replaced by amino groups. Substitution of the hemiacetal hydroxyl group by an amino group yields ***N*-glycosides** called **glycosylamines**. Aldoses produce aldosylamines (**4-99**, R = H) and ketoses ketosylamines (**4-100**). Various glycosylamines are natural components of all foods. Common compounds are nucleosides, nucleotides (such as ATP) and their polymers RNA and DNA that contain D-ribose or 2-deoxy-D-ribose, respectively. Glycosylamines also form as primary products of the Maillard reaction and in reactions of sugars with ammonia,



4-99, β-D-glucosylamine

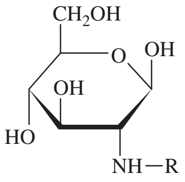


4-100, β-D-fructosylamine

respectively. With amines, amino acids and proteins, sugars yield *N*-substituted glycosylamines and reactions of glycosylamines with another molecule of sugar give diglycosylamines. Glycosylamines are generally unstable basic compounds that are hydrolysed by acids to the parent sugars and amino compounds, rearranged to aminodeoxysugars or readily decomposed.

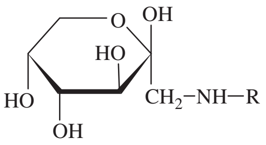
Substitution of a group other than the hemiacetal hydroxyl group for an amino group yields aminodeoxy derivatives of monosaccharides, which are commonly called **aminodeoxysugars** or **amino sugars** for short. The prefix amino in the nomenclature indicates the replacement of a hydrogen atom, therefore aminosugars are derivatives of deoxysugars. Aminosugars are generally known as glycosamines, aminodeoxysugars derived from hexoses are called **hexosamines**, aminodeoxysugars derived from aldoses are **aldosamines** and **ketosamines** are derived from ketoses. Aminosugars derived from aldoses commonly have the amino group on carbon C-2 and amino sugars derived from ketoses on carbon C-1. Amino sugars are unstable as free bases and decompose to a variety of products. Their salts (such as hydrochlorides) are more stable compounds.

Aldosamines and their *N*-acetyl derivatives are important components of various biologically active oligosaccharides and biopolymers. The most important aldosamines are D-glucosamine, D-galactosamine and D-mannosamine. D-Glucosamine is also known as D-chitosamine (2-amino-2-deoxy-D-glucose, **4-101**, R = H). *N*-Acetyl-D-glucosamine (**4-101**, R = $COCH_3$) is a structural unit of milk oligosaccharides, of polysaccharide chitin and of numerous heteropolysaccharides found in proteoglycans of connective tissue, in synovial fluids, in joints connecting bones, in respiratory tract mucus, in saliva, in glycoproteins of milk, eggs and blood serum and in peptidoglycans of bacterial cells. D-Galactosamine also known as D-chondrosamine (2-amino-2-deoxy-D-galactose) is in the form of the *N*-acetate (*N*-acetyl-D-galactosamine, R = $COCH_3$), the building unit of milk oligosaccharides occurring in various connective tissue proteoglycans (mucoproteins) and in glycoproteins of milk and eggs. D-Mannosamine is, as *N*-acetyl-D-mannosamine, a precursor of acetylneuraminic acid.



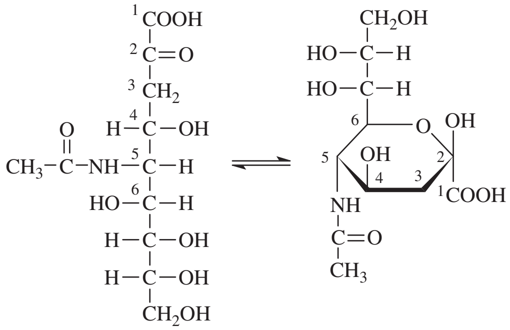
4-101, β-D-glucosamine

Ketosamines, also known as 1-amino-1-deoxyketoses, have an analogous structure (**4-102**). Some important ketosamines form as intermediates of the Maillard reaction.



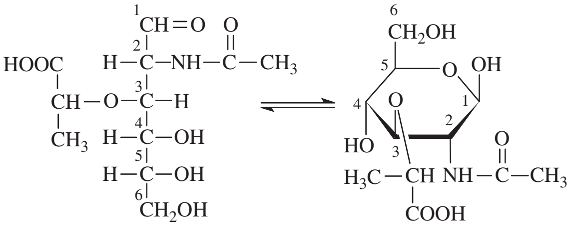
4-102, β-D-fructosamine

Biochemically important amino derivatives of sugars that are widespread in animal connective tissues (mainly in glycoproteins and gangliosides) and microorganisms are **sialic acids**. Sialic acids are a group of α-oxoacids, which are formed by condensation of *N*-acetylhexosamines with three carbon-carboxylic acids. About 50 sialic acids are known. They are *N*- or *O*-substituted derivatives of neuraminic (5-amino-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic) acid (abbreviated as Neu), a monosaccharide with a nine-carbon backbone that does not occur in nature. The neuraminic acid amino group can be acylated by acetic or glycolic acid and hydroxyl groups, except for the semiacetal hydroxyl, can be esterified with lactic, sulfuric or phosphoric acids or methylated. The most common compound, that is generally widespread, is *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid, also known as the *N*-acetyllactaminic acid and as Neu5Ac for short) (**4-103**), which is biosynthesised from *N*-acetyl-D-mannosamine and pyruvic acid. The cyclic form of this acid (α-anomer), commonly called *N*-acetyl-α-neuraminic acid, is a constituent of many minor milk oligosaccharides and, together with the corresponding *N*-glycolyl derivative, occurs in numerous glycoproteins (e.g. in milk and egg white glycoproteins). Examples of other sialic acids are *N*-glycolylneuraminic acid (Neu5Gc) and *N*-acetyl-9-*O*-acetylneuraminic acid (Neu5,9Ac2), which, however, do not occur in all living organisms.



4-103, *N*-acetylneuraminic acid

Structurally related to sialic acids is *N*-acetylmuramic acid (**4-104**), the building unit of peptidoglycans of bacterial cell walls or **mureins**.



4-104, *N*-acetylmuramic acid

4.4 Oligosaccharides

Oligosaccharides include those oligomers of monosaccharides in which 2–10 molecules of monosaccharides are connected with each other through glycosidic linkages. Oligosaccharides are therefore glycosides in which the aglycone is a molecule of another saccharide. They are therefore homoglycosides. Depending on the number of monose units, oligosaccharides are divided into di-, tri-, tetra-, penta- and up to decasaccharides. Monosaccharides in oligosaccharides may occur in the form of pyranoses or furanoses. The most common monosaccharides found are hexoses.

Disaccharide can theoretically be formed by the condensation of the α- or β-anomeric hydroxyl group of a monosaccharide with any hydroxyl group of another monosaccharide. The biosynthesis of glycosides, oligosaccharides and polysaccharides but requires the activation of sugar (its 1-phosphate) by binding to nucleoside bisphosphate. The active forms of glucose, for example, are adenosine diphospho-α-D-glucopyranose, guanidine diphospho-α-D-glucopyranose and uridine diphospho-α-D-glucopyranose. The condensation of two semiacetal hydroxyls then yields a disaccharide that does not contain a free anomeric hydroxyl group and is therefore non-reducing. In any other cases, the condensation of two monosaccharides gives a reducing disaccharide, which like the parent monosaccharides exhibits mutarotation in solution, and thus occurs as the α- or β-anomer. Connecting monosaccharides to disaccharides generates trisaccharides, and subsequently higher oligosaccharides.

As an example, Figure 4.5 lists all the hypothetical condensation products of α-D-glucopyranose and β-D-glucopyranose via the anomeric hydroxyl groups with hydroxyls in position C-4. The condensation of two molecules of α-D-glucopyranose through the semiacetal hydroxyl groups yields non-reducing disaccharide known as α,α-trehalose (Figure 4.5). The condensation of the semiacetal hydroxyl of one molecule with the hydroxyl group of the second molecule of α-D-glucopyranose at C-4 gives reducing disaccharide maltose (Figure 4.5), its α-anomer, which yields a certain amount of β-anomer by mutarotation (the rate of convergence to equilibration depends mainly on pH of the reaction medium).

Maltose is also produced by condensation of the anomeric hydroxyl of α-D-glucopyranose with the hydroxyl at C-4 of β-D-glucopyranose when mutarotation again gives the equilibrium mixture of α- and β-anomers of maltose. Condensation of both semiacetal hydroxyl groups yields α,β-trehalose, known as neotrehalose (Figure 4.5). Condensation of the anomeric

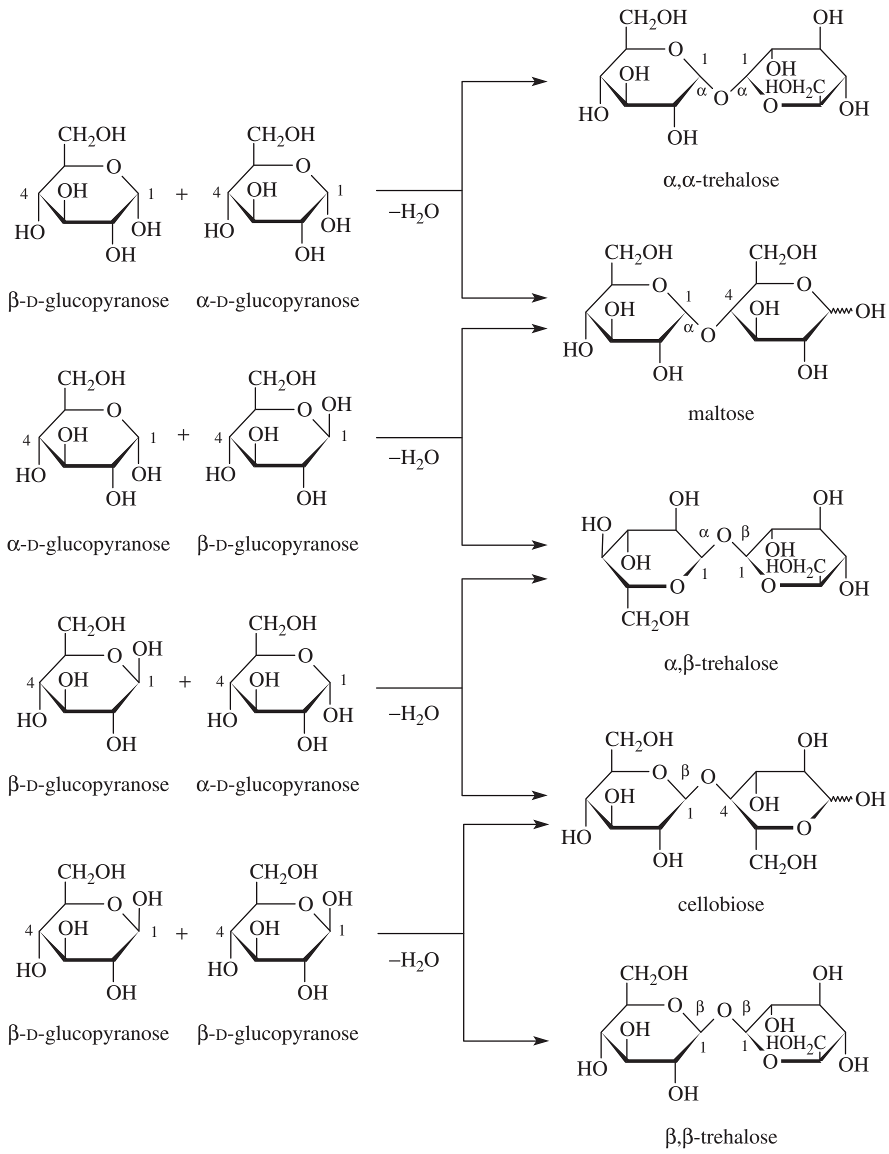


Figure 4.5 Products of condensation of α-D-glucopyranose and β-D-glucopyranose.

hydroxyl of β-D-glucopyranose with the hydroxyl at C-4 of α- or β-D-glucopyranose gives the reducing disaccharide cellobiose (Figure 4.5). Condensation of both anomeric hydroxyls of β-D-glucopyranose gives β,β-trehalose, known as isotrehalose (Figure 4.5). Eight other reducing sugars can be formed by condensation of the anomeric hydroxyl groups of α-D-glucopyranose and β-D-glucopyranose with hydroxyl groups at C-2, C-3 and C-6 of another molecule of D-glucose (see Table 4.11).

In the nomenclature of oligosaccharides, trivial names are still used for a number of substances (e.g. maltose, cellobiose or trehalose, Figure 4.5). In the case of reducing disaccharides, systematic nomenclature takes as its basis the name of the monosaccharide with a free hemiacetal hydroxyl group, which is preceded by the name of the substituted monosaccharide and the respective anomeric configuration is indicated (α- or β-). Disaccharide maltose (Figure 4.5), formed by condensation of the anomeric hydroxyl group of α-D-glucopyranose with the C-4 hydroxyl group of another molecule of D-glucose is generally **glycosylglycose** and is referred to as *O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose. The previously used name was 4-*O*-α-D-glucopyranosyl-D-glucopyranose. The molecule of α-D-glucopyranose is bound by the hemiacetal hydroxyl group (at carbon C-1) and the *O*-glycoside to the hydroxyl group at C-4 (which is marked with numbers 1→4) to the second molecule of D-glucopyranose (α- or β-). In the case of the α-anomer of maltose, the trivial name α-maltose or the systematic name *O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranose can be used. The abbreviated name of maltose can be written as follows: α-D-Glc*p*-(1→4)-D-Glc*p* (abbreviated names of oligosaccharides are formed from trivial names of monosaccharide units). The systematic name of cellobiose (Figure 4.5) is *O*-β-D-glucopyranosyl-(1→4)-D-glucopyranose, for short β-D-Glc*p*-(1→4)-D-Glc*p*.

Table 4.11 Overview of important glucooligosaccharides.

Trivial name	Abbreviated notation	Occurrence
Disaccharides		
Trehalose (α,α-trehalose)	α-D-Glc*p*-(1↔1)-α-D-Glc*p*	Yeast, mushrooms, honey
Neotrehalose (α,β-trehalose)	α-D-Glc*p*-(1↔1)-β-D-Glc*p*	Honey, koji[a]
Isotrehalose (β,β-trehalose)	β-D-Glc*p*-(1↔1)-β-D-Glc*p*	Honey, glucose reversion
Kojibiose	α-D-Glc*p*-(1→2)-D-Glc*p*	Honey, koji[a]
Nigerose (sakebiose)	α-D-Glc*p*-(1→3)-D-Glc*p*	Honey, beer
Maltose	α-D-Glc*p*-(1→4)-D-Glc*p*	Starch structural unit, starch syrup, honey, malt, sugar beet
Isomaltose (brachiose)	α-D-Glc*p*-(1→6)-D-Glc*p*	Starch structural unit, starch syrup, honey
Sophorose	β-D-Glc*p*-(1→2)-D-Glc*p*	Glucose reversion, glycosides
Laminaribiose	β-D-Glc*p*-(1→3)-D-Glc*p*	Honey
Cellobiose	β-D-Glc*p*-(1→4)-D-Glc*p*	Cellulose structural unit, honey
Gentiobiose	β-D-Glc*p*-(1→6)-D-Glc*p*	Honey, glycosides
Trisaccharides		
Maltotriose	α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*	Starch syrup, honey
Cellotriose	β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-D-Glc*p*	Cellulose degradation
3-α-isomaltosylglucose	α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→3)-D-Glc*p*	Honey
Isomaltotriose (dextrantriose)	α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-D-Glc*p*	Honey
Centose	α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→2)-D-Glc*p*	Honey
Panose	α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p*	Amylopectin degradation, honey
Isopanose	α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→6)-D-Glc*p*	Honey
Neapolitanose	β-D-Glc*p*-(1→2)-[β-D-Glc*p*-(1→6)]-D-Glc*p*	Glycosides
Tetrasaccharides		
Maltotetraose	α-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→4)]$_2$-D-Glc*p*	Starch syrup
Isomaltotetraose	α-D-Glc*p*-(1→6)-[α-D-Glc*p*-(1→6)]$_2$-D-Glc*p*	Honey

[a]Koji is a mixture of soybean meal and wheat meal fermented by fungi *Aspergillus oryzae* and *A. Soyae*. It is an intermediate in the production of soy sauce (shoyu).

Cellobiose, as well as maltose, is a reducing sugar and therefore occurs in the form of α- and β-anomers.

Non-reducing disaccharides are actually double glycosides, and are therefore called **glycosylglycosides**. Disaccharide α,α-trehalose (Figure 4.5) is α-D-glucopyranosyl-α-D-glucopyranoside for short: α-D-Glc*p*-(1↔1)- α-D-Glc*p*.

Disaccharides and higher oligosaccharides exist in the form of different conformational isomers, which differ by the energy state given by the rotation of molecules of monosaccharides around the glycosidic bond, therefore the conformational isomers differ in their various spatial orientations. They can be characterised as peptides by torsion angles ϕ and ψ (see Section 2.3.1). Equilibrium at a given temperature exists for the populations of individual conformers. The predominant species are the conformers with the lower free energy content. Most oligosaccharides have the same stable and sterically most favourable conformers in both aqueous solutions and in the solid (crystal) state. Conformers of disaccharides are commonly stabilised by hydrogen bonds between adjacent hydroxyl groups or between hydroxyl groups and the oxygen atom of pyran or furan rings.

Foods (such as fruits, vegetables, milk and honey) contain a large number of free and bound oligosaccharides, which are natural food ingredients. Oligosaccharides are usually composed of a number of common monosaccharides of the D-series (glucose, fructose, galactose and mannose) in various combinations. Aldoses are present in the form of pyranoses and fructose as furanose. Less common sugars, such as 6-deoxyhexose (methylpentose) L-rhamnose or pentoses L-arabinose and D-xylose are also present in some oligosaccharides.

The most common oligosaccharides present as natural components of foods are oligomers of D-glucose known as **glucooligosaccharides**, in which glucose is the sole or

predominant monosaccharide. Another important group are the **fructooligosaccharides** containing only D-fructose or D-fructose and D-glucose. The dominant disaccharide of this type in foods is saccharose (sucrose). Equally important are **galactooligosaccharides**, which consist of D-galactose, D-glucose, D-fructose and may contain also some other monosaccharides.

The human organism can hydrolyse some oligosaccharides, such as saccharose, lactose, maltose and isomaltose-type oligosaccharides, to monosaccharides by the hydrolytic enzymes maltase, isomaltase, saccharase (sucrase) and lactase (see Section 4.2.3). Utilisable oligosaccharides have a similar effect on blood glucose level as the corresponding monosaccharides. Saccharose and maltose exhibit strong cariogenic effects, while the effects of lactose are weaker. Lactose has some laxative effects.

In recent years, a number of various oligosaccharides have been produced and used as food additives. Raw materials for edible oligosaccharides are naturally present oligosaccharides (e.g. saccharose and lactose) and polysaccharides (starch, inulins, xylans and other polysaccharides). Currently, more than ten different types of oligosaccharides are produced. For example, saccharose is a raw material for the production of palatinose (isomaltulose), glycosylsaccharose, lactosaccharose and fructooligosaccharides. Lactulose, lactosaccharose and galactooligosaccharides are produced from lactose. Starch is the raw material for the production of maltooligosaccharides, isomaltooligosaccharides, cyclodextrins and gentiooligosaccharides. Fructooligosaccharides are produced from inulin, xylooligosaccharides from xylans and mannooligosaccharides from mannose. Hydrogenation of some disaccharides (lactose and maltose) yields the corresponding polyols. The oligosaccharides (and polyols) obtained are water-soluble substances having a slightly sweet taste. Their solutions usually have a higher viscosity than solutions of natural monosaccharides and disaccharides and only 30–60% of the sweetness of saccharose. These sugars therefore find use as fillers for low-energy products (with reduced monosaccharide and saccharose content), for products that require increased viscosity and increased water binding capacity as a prevention of drying. For example, lower water activity of products is a protection against microbial contamination and starch retrogradation during bread aging.

The physiological effects of these oligosaccharides are highly desirable. Unlike ordinary monosaccharides, oligosaccharides and starch, these oligosaccharides are not utilised by microflora of the mouth and are therefore non-cariogenic carbohydrates. Many of them are indigestible and cannot be utilised, and are used in the production of low energy foods and foods for diabetics. Some oligosaccharides exhibit:

- **prebiotic** effects, as they selectively stimulate the growth and metabolism of desirable microflora of the colon
- **probiotic** effects, as the desirable microflora confers health benefits on the host
- **symbiotic** effects (prebiotic and probiotic effects at the same time).

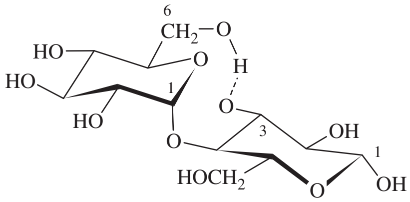
4.4.1 Glucooligosaccharides

Glucooligosaccharides include a large number of disaccharides, trisaccharides and higher oligosaccharides, which commonly (but usually in small quantities) occur in many foods or are used as food additives (Table 4.11). The most important representative of these oligosaccharides is the disaccharide maltose.

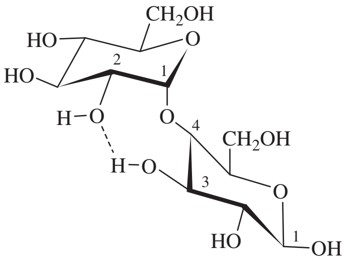
4.4.1.1 Maltose

4.4.1.1.1 Structure and nomenclature

A molecule of the reducing sugar maltose (also called malt sugar, as it is the result of starch hydrolysis in malt), known by the systematic name *O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose (Figure 4.5), is stabilised in aqueous solution by hydrogen bonds between the hydroxymethyl group of one molecule of glucose (C-6) and the hydroxyl group at carbon C-3 of the second molecule of glucose (**4-105**). In crystals of maltose (and also in solutions in non-aqueous media), maltose is present as a different conformer, which is stabilised by hydrogen bonds between the hydroxy group at the C-2 carbon of one glucose molecule and the hydroxyl at the C-3 of the second molecule of glucose (**4-106**).



4-105, conformation of maltose in aqueous solutions



4-106, conformation of anhydrous crystalline maltose

4.4.1.1.2 Occurrence

Small, but sometimes also relatively large, amounts of maltose are typically found in most foods. Bread dough contains maltose formed by hydrolysis of starch by yeast (*Saccharomyces cerevisiae*) enzymes and by enzymes present in the flour. At the beginning of the fermentation, its content increases due to the hydrolysis of starch. After the preferred fermentation of glucose and fructose, the maltose content decreases, as it is also partially fermented by yeast. The level of maltose present in bread is 1.7–4.3%.

Maltose, a product of the enzymatic hydrolysis of starch, is present in germinating seeds and thus also in germinating barley

and barley malt (hence the name malt sugar). Malt may be partly substituted by starch-rich materials, such as rice, maize or wheat. Brewer's yeast ferments about 95% of maltose and 80% of maltotriose.

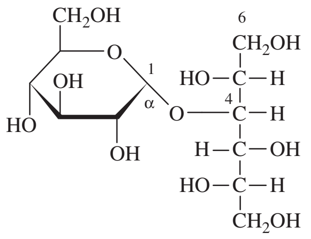
Maltose is present at a relatively large concentration in honey (2.7–16%, 7.3% on average) together with fructose (27.3–44.3%), glucose (22.0–40.8%), saccharose (0.3–7.6%) and other oligosaccharides. It is also found in cereals and fruits. Starch hydrolysates, such as maltose syrups, contain maltose at levels of up to 85%.

4.4.1.1.3 Physiology and nutrition

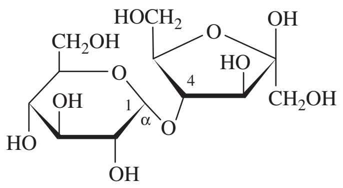
The relative sweetness of maltose is 30–60% of saccharose sweetness (see Table 8.37). After hydrolysis to glucose by maltase (α-D-glucoside glucohydrolase), maltose is a utilisable sugar. Maltose intake in foods has a significant effect on blood glucose levels and insulin secretion. It is a cariogenic sugar.

4.4.1.1.4 Use

Maltose and various products containing maltose and maltooligosaccharides (such as maltodextrins, glucose or maltose syrups) are obtained from starch, often by the combined effects of mineral acids and amylolytic enzymes. Maltose is obtained from maltose syrups. A product of maltose hydrogenation (at the reducing end of the glucose molecule) is maltitol, *O*-α-D-glucopyranosyl-(1→4)-D-glucitol (**4-107**). Its sweetening power is about 90% of that of saccharose. Maltitol has very little impact on blood sugar level and has a weak laxative effect. Isomerisation in an alkaline medium (glucose at the reducing end of the molecule isomerises to fructose) produces disaccharide maltulose (**4-108**).



4-107, maltitol



4-108, maltulose

4.4.1.2 Other glucooligosaccharides

An overview of common glucooligosaccharides occurring in foods is given in Table 4.11. Many different glucooligosaccharides are present in honey as minor sugars (Table 4.12). Their structures are shown in Table 4.11; the structures of fructooligosaccharides are shown later in Table 4.14.

Table 4.12 Composition of minor disaccharides of honey.

Disaccharides	Content (g/kg)	Trisaccharides	Content (g/kg)
Kojibiose	3.0	Erlose	1.6
Turanose	1.7	Theanderose	0.99
Isomaltose	1.6	Panose	0.91
Saccharose	1.4	Maltotriose	0.69
Maltulose	1.1	1-Kestose	0.33
Nigerose	0.62	Isomaltotriose	0.22
Isotrehalose	0.40	Melezitose	0.11
Gentiobiose	0.15	Isopanose	0.09
Laminaribiose	0.03	Centose	0.02

An important non-reducing disaccharide is α,α-trehalose, which occurs in many plants and invertebrate organisms (bacteria, fungi, worms, crustaceans), where it serves as an energy source and has a protective function in stress induced by heat and drought. In fruits and vegetables, trehalose is found in very small amounts (see Table 4.5), but larger levels are located in fungi. In the cultivated common mushroom (*Agaricus bisporus*), the content of α,α-trehalose is 0.5% in very young fruiting bodies and 0.09% in fruiting bodies at the end of their development, but it constitutes 21% of dry matter in the charbonnier mushroom (*Tricholoma portentosum*).

Numerous disaccharides and higher oligosaccharides containing glucose, in addition to other monosaccharides, are components of many glycosides. Common sugars occurring in glycosides are arabinose, xylose and rhamnose. An overview of the major reducing disaccharides occurring in anthocyanins, other flavonoids and other glycosides is shown in Table 4.13.

4.4.2 Fructooligosaccharides

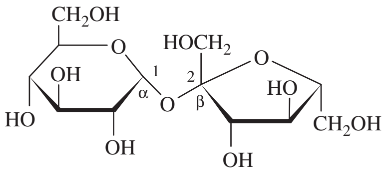
4.4.2.1 Saccharose

4.4.2.1.1 Structure and nomenclature

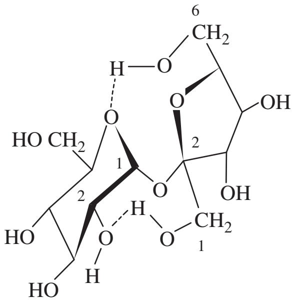
Saccharose or sucrose (**4-109**), β-D-fructofuranosyl-α-D-glucopyranoside (sugars are given in alphabetical order), also called beet, cane or table sugar, is the most important representative of non-reducing disaccharides. In aqueous solutions and in the solid state, the conformer stabilised by two hydrogen bonds predominates (**4-110**). The first bond is between the hydroxyl group on carbon C-2 of glucose ($^4C_1$-pyranose) and the hydroxyl group at C-1 of fructose ($^4T_3$-furanose), the second bond is between the pyranose ring oxygen and the C-6 hydroxyl group of the fructose.

Table 4.13 Important disaccharides occurring in glycosides.

Trivial name	Short notation	Trivial name	Short notation
Vicianose	β-L-Ara*p*-(1→6)-D-Glc*p*	Robinobiose	α-L-Rha*p*-(1→3)-D-Glc*p*
Sambubiose	β-D-Xyl*p*-(1→2)-D-Glc*p*	Chacobiose	α-L-Rha*p*-(1→4)-D-Glc*p*
Primeverose	β-D-Xyl*p*-(1→6)-D-Glc*p*	Rutinose	α-L-Rha*p*-(1→6)-D-Glc*p*
Neohesperidose	α-L-Rha*p*-(1→2)-D-Glc*p*	Solabiose	β-D-Glc*p*-(1→3)-β-D-Gal*p*



4-109, saccharose



4-110, conformation of saccharose in aqueous solutions

4.4.2.1.2 Occurrence and production

Saccharose is a very widespread sugar synthesised by most eukaryotic and some prokaryotic organisms. As the main product of photosynthesis, saccharose is commonly present in higher plants, where it has a role in transport and energy reserves and is used also in signal transmission and in stress situations. As the energy reserve in plants, saccharose is mobilised under the catalysis of the enzyme invertase to fructose and glucose and is used for seed germination, plant growth and also during fruit ripening. Another enzyme that plays a role in the catabolism of sucrose is sucrose synthetase, which splits sucrose into fructose and UDP-α-D-glucose, which is used for the biosynthesis of starch.

Saccharose is present in larger amounts in the vegetative parts of plants, such as leaves and stems (sugar cane contains 12–26%, sugar-rich varieties called sweet maize 12–17%, sweet millet 7–15% sucrose) and fruits (e.g. apples, oranges, apricots, peaches, pineapples and papayas), which contain up to about 8% saccharose (Table 4.4). Some fruits, however, do not contain sucrose (cherries, grapes, figs), because it is hydrolysed during fruit ripening. Vegetables commonly contain 0.1–12% saccharose (e.g. onion 10–11% and in sugar beets 3–20%). Mustard seeds, rapeseeds and other oilseeds contain about 4%, wheat flour from 0.1 to 0.4%, green coffee 6–7% (roasted coffee about 0.2%) and groundnuts 4–12 % saccharose. Sucrose is the transport form from the leaves to the tubers and is therefore the major sugar in immature potato tubers. The amount of saccharose in mature tubers varies between cultivars and is generally less than 0.3%. If saccharose is low in tubers at harvest, the concentration of all sugars remains low in healthy tubers under storage. However, the content of saccharose in Jerusalem artichokes is 2–3%. The saccharose content in beans is given in Table 4.17.

The main industrial sources of sucrose are sugar cane (*Saccharum officinarum*, Poaceae) and sugar beet (*Beta vulgaris* group *Altissima*, Amaranthaceae, formerly Chenopodiaceae), the varieties of which today contain 15–20% saccharose (typically 16–17%). In addition to sugar cane and sugar beet, on smaller scale and locally, other plants are sometimes used as a source of saccharose. In Algeria and Iraq, date sugar is obtained from fruits of date palm dates (*Phoenix dactylifera*, Arecaceae) that contain up to 81% saccharose in the dry state. In India, Cambodia, the Philippines and elsewhere, table sugar is traditionally made from the juice of various palms of the genus *Borassus* (e.g. *Borassus flabelliformis*) and of some other species, such as arenga palm (*Arenga pinnata*), date palm and coconut palm (*Cocos nucifera*). Maple sugar in Canada, the United States and Japan is produced from the juice of sugar maple (*Acer saccharum*, Sapindaceae), which contains about 5% saccharose. Sweet sorghum syrup, traditionally used as a substitute for sugar, is known as molasses, especially in Southern US states, although it is not true molasses. It is obtained from the stalks of sweet sorghum (*Sorghum bicolor*, Poaceae) containing 12% saccharose.

The production of saccharose from sugar beet consists of several steps, including **diffusion**, juice **purification**, **evaporation** and **crystallisation**. The processing of thoroughly washed sugar beet starts by slicing the beets into thin chips called **cossettes**. The cossettes are extracted with hot water at an elevated temperature (50–80 °C) in a process called **diffusion** (sulfur dioxide, chlorine, ammonium bisulfite or commercial biocides are used as disinfectants). The sugar-enriched water, called **raw juice** (or mixed juice containing between 10 and 15% of saccharose) is filtered, heated to 80–85 °C and cleaned by a process known as **carbonatation**, which removes non-sucrose impurities in the raw juice. Lime milk (a solution of calcium hydroxide) is added to adsorb or adhere to the impurities, and carbon dioxide gas is bubbled through the mixture to precipitate the lime as insoluble calcium carbonate crystals (see Section 6.3.2.3). After filtration (the liming processes with

subsequent carbonation and filtration are known as **epuration**), sulfur dioxide is added to the juice (termed **thin juice**) to inhibit the Maillard reaction. The next stage of the process is the juice **evaporation** to **thick juice** that contains 50–67% saccharose. The filtered juice with added pure sugar, or **standard liquor**, proceeds to the **crystallisation**. It is boiled under vacuum until it becomes supersaturated and saccharose crystallises. Sugar crystals in the mixture with liquor, known as **massecuite** (or fillmass), are separated and refined. Raw sugar obtained by crystallisation contains about 96% saccharose, 1.0–1.2% organic compounds, 0.8–1.0% inorganic substances (so called non-sugars) and 1–2% water. It is light yellow to dark brown because it contains a certain amount of molasses. It can be used as raw brown sugar. The refining is mainly done with hot water, the product is dried and cooled. The liquid separated from the sugar crystals, the **syrup**, is introduced back to the crystallisation operation; the process is repeated once again so that the yield of saccharose is 58–90%.

The final liquid obtained is **molasses**. Beet molasses contains about 50% sucrose and about 80–85% dry matter, from which about 1% accounts for glucose and fructose and 1.2% (or it can sometimes be more than 8%) for raffinose. In small quantities, some other oligosaccharides also occur. Approximately 20% of the molasses weight is accounted for by non-sugars consisting of organic acids and amino acids (particularly glutamic acid, 5-oxopyrrolidine-2-carboxylic acid) and *N*,*N*,*N*-trimethylammonioacetate (betaine, **2-14**), the content of which is generally 0.3%, and other substances. The rest (about 12%) is accounted for by inorganic substances such as potassium salts of sugars and other substances. Molasses can be further desugarised using an ion-exchange process called deep molasses desugarisation, used as a substrate for the production of yeast, ethanol, citric and lactic acid, glycerol, butan-1-ol, acetone and many other substances, or used in the production of livestock feed or for a range of other purposes. The pressed beet pulp (cossettes) is mixed with molasses that is not desugarised, then dried and used as a constituent of some animal feeds.

Sugar cane molasses contains about 80% dry matter, of which 30–40% is saccharose and 30% glucose and fructose. The content of non-sugars is about 10%. The non-sugars include small amounts of raffinose, no betaine, but also about 5% aconitic acid, which is not present in beet molasses. The content of inorganic substances is about 8%. Sugar cane molasses is mainly used for production of rum and arrack.

4.4.2.1.3 Physiology and nutrition

Saccharose occurring in foods is hydrolysed to glucose and fructose by invertase, β-D-fructofuranosidase; therefore it has a large influence on the glucose content in plasma and insulin secretion. A high sucrose intake causes a significant excess of energy and the body synthesises higher amounts of fat. Saccharose is a cariogenic sugar.

4.4.2.1.4 Use

Saccharose is predominantly used as a versatile sweetener and is also used in the production of:

- invert sugar
- fructooligosaccharides (also produced from inulin)
- palatinose and palatinitol
- glycosylsaccharose
- lactosaccharose (also produced from lactose).

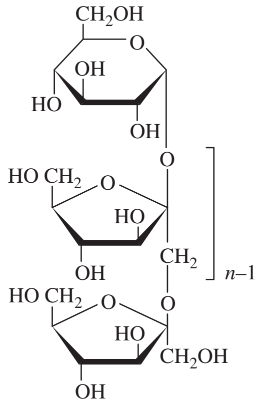
Invert sugar

Acid or enzymatic hydrolysis of sucrose, **inversion**, produces an equimolar mixture of D-glucose and D-fructose known as invert sugar. Invert sugar is used as a food additive substance, usually in the form of syrup (relative sweetness is 95–105% of saccharose sweetness). It also serves as a starting material for obtaining D-glucose and D-fructose, the sweeteners mannitol and glucitol and other substances.

Invert sugar obtained by acid hydrolysis of saccharose contains trace amounts of reversion products of glucose (especially isomaltose and gentiobiose) and fructose (dianhydrides or laevulosans). Invert sugar obtained by enzymatic hydrolysis (due to some transglucosidase or transfructosidase activity of invertase) also contains trace amounts of some less common oligosaccharides.

Fructooligosaccharides

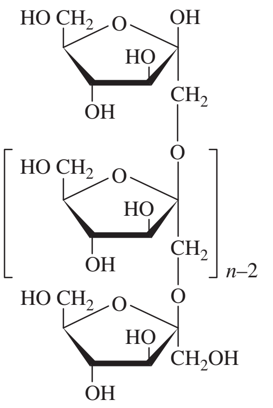
Fructooligosaccharides (**4-111**) are produced from saccharose by the action of microbial and plant fructosyltransferase (transfructosylation) or by using the transfructosylase activity of invertase. Fructooligosaccharides are oligomers of the type: [β-D-Fru*f*-(2→1)]$_{n-1}$-β-D-Fru*f*-(2↔1)-α-D-Glc*p*, for short GF_n (G = glucose, F = fructose, *n* = degree of polymerisation). The basic member of the homologous series is saccharose and the degree of polymerisation is 2–4. This mixture of fructooligosaccharides is known as **neosugar**.



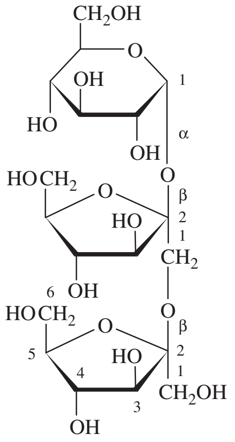
4-111, type GF_n fructooligosaccharides

Fructooligosaccharides of somewhat different composition are produced by a controlled enzymatic hydrolysis of inulin

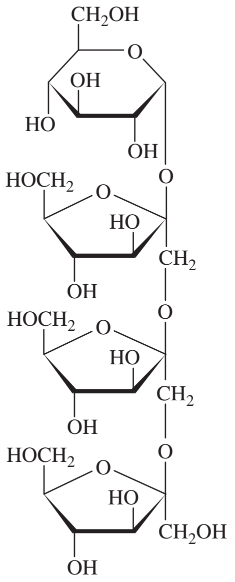
using β-D-fructofuranosidase (invertase, saccharase) from the fungus *Aspergillus niger*. The hydrolysis products are fructooligosaccharides of the type: [β-D-Fru*f*-(2→1)]$_{n-1}$-β-D-Fru*f*-(2↔1)-α-D-Glc*p*, abbreviated GF_n, or products of the type: [β-D-Fru*f*-(2→1)]$_n$, in short F_n, where $n = 2$–9. These products are called **oligofructose** (**4-112**). The basic member of the fructooligosaccharides of the GF_n type is the non-reducing trisaccharide 1-kestose (**4-113**), abbreviated to GF_2. Higher oligosaccharides are tetrasaccharide 1,1-nystose (GF_3, **4-114**) and pentasaccharide 1-*O*-β-D-fructofuranosylnystose (1,1,1,-fructosylnystose, GF_4). The GF_n type oligomers are non-reducing sugars. An example of disaccharides consisting of two molecules of fructose is inulobiose (**4-115**), abbreviated to F_2. The F_n type oligomers, which contain glucose, are reducing sugars.



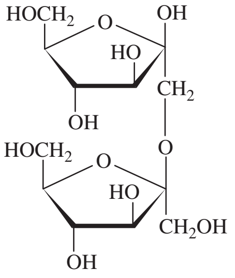
4-112, type F_n fructooligosaccharide



4-113, 1-kestose



4-114, 1,1-nystose

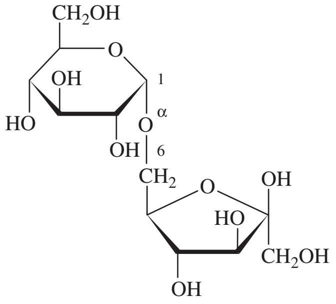


4-115, inulobiose

Fructooligosaccharides are water-soluble sweet substances, which show 40–60% of the saccharose sweetness. They are not hydrolysed by saccharidases and therefore are classified as soluble fibre. In the colon, however, fructooligosaccharides are fermented by anaerobic bacteria to lower fatty acids (mainly acetic, propionic and butyric acids), L-lactic acid and gases (carbon dioxide, methane and hydrogen). They are therefore also called intestinal food. The yield of energy of fructose bound in oligofructoses is only about 25–35% of the energy yield of free fructose (about 4.2–6.3 kJ/g). Fructooligosaccharides are the growth factor of beneficial bifidobacteria (*Bifidobacterium bifidum*). They produce carboxylic acids (mainly acetic acid and lactic acid) and the lower pH is associated with the production of substances with antibiotic and immunomodulatory effects (so-called bifidin and other substances), which results in suppression of the growth of undesirable microflora, such as *Escherichia coli*, *Streptococcus faecalis*, *S. proteus*, *Clostridium perfringens* bacteria and also *Staphylococcus aureus*, *Salmonella typhosa* and some other bacteria, which produce toxic fermentation products (ammonia, amines, nitrosamines, phenols, indoles and other toxins). In addition, bifidobacteria produce thiamin, riboflavin, niacin, pyridoxine, folacin and corrinoids. Fructooligosaccharides and the so-called soluble fibre bind bile acids in the intestine very well, and thus prevent their reabsorption, which is manifested by lowering of the cholesterol level in blood plasma.

Palatinose

Isomerisation of saccharose using immobilised microorganisms *Leuconostoc mesenteroides* or *Protaminobacter rubrum* yields palatinose, also known as isomaltulose (**4-116**, Table 4.14). The ratio of α- and β-anomers of isomaltulose (usually present in the ratio 1 : 4) depends on the reaction conditions. Palatinose is accompanied by small amount of α-D-fructofuranosyl-(1↔1)-α-D-glucopyranoside. Intermolecular condensation of these disaccharides yields higher oligosaccharides. Palatinose is a non-cariogenic sugar that stimulates the growth of bifidogenic microflora. The sweetening power of palatinose is about 40% of the sweetening power of saccharose.



4-116, isomaltulose

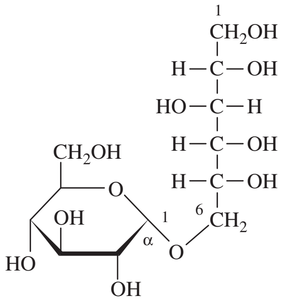
Palatinitol

Catalytic hydrogenation of palatinose yields palatinitol, which is known by the trade name of **isomalt**. Isomalt is a mixture of *O*-α-D-glucopyranosyl-(1→6)-D-glucitol (isomaltitol, **4-117**) and *O*-α-D-glucopyranosyl-(1→1)-D-mannitol in a ratio of approximately 1 : 1. The sweetening power of this non-cariogenic sugar is about

Table 4.14 Overview of important fructooligosaccharides.

Trivial name	Abbreviated notation	Occurrence
Disaccharides		
Saccharose	β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Honey, sugar beet, sugar cane plants
Turanose	α-D-Glc*p*-(1→3)-D-Fru*f*	Honey
Maltulose	α-D-Glc*p*-(1→4)-D-Fru*f*	Honey, maltose reversion
Isomaltulose (palatinose)	α-D-Glc*p*-(1→6)-D-Fru*f*	Maltose reversion, saccharose isomerisation
Inulobiose	β-D-Fru*f*-(2→1)-β-D-Fru*f*	Inulin degradation
Levanbiose	β-D-Fru*f*-(2→6)-β-D-Fru*f*	Inulin degradation
Trisaccharides		
1-Kestose (isokestose)	β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Honey, inulin, invert sugar, saccharases from saccharose
6-Kestose (kestose)	β-D-Fru*f*-(2→6)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Bananas, honey, invert sugar, molasses
Neokestose	β-D-Fru*f*-(2→6)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	Inulin, invert sugar,saccharases from saccharose
Erlose	α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	Honey
Theanderose	α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	Honey
Gentianose	β-D-Glc*p*-(1→6)-α-D-Glc*p*-(1↔2)-β-D-Fru*f*	Glycosides
Melezitose	α-D-Glc*p*-(1→3)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Nectars, honey
Kelose	α-D-Fru*f*-(2→6)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Saccharases from saccharose
Tetrasaccharides		
Nystose	β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2→1)-β-D-Fru*f*-(2↔1)-α-D-Glc*p*	Inulin, saccharases from saccharose

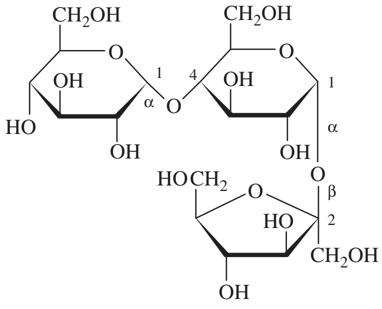
50% of the sweetening power of saccharose. It has no effect on blood glucose level, but it is relatively effective laxative.



4-117, isomaltitol

Glycosylsaccharose

Trisaccharide glycosylsaccharose (**4-118**) is produced under the trade name **neosugar** from saccharose or maltose using the enzyme cyclomaltodextrin glucanotransferase. It has about 50% of the sweetening power of sucrose, is less digestible and non-cariogenic. It is appreciated for its technological properties, such as reduced crystallisation of saccharose, reduced retrogradation of starch and low reactivity in the Maillard reaction.



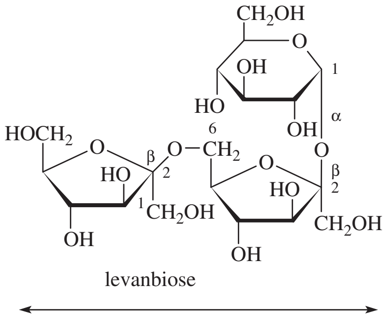
4-118, glycosylsaccharose

4.4.2.2 Other fructooligosaccharides

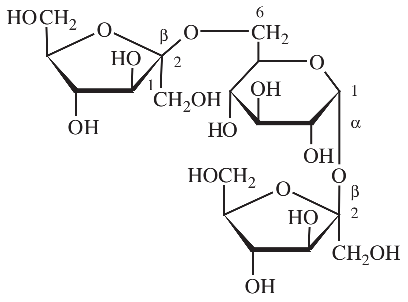
In addition to saccharose, numerous other disaccharides and higher oligosaccharides derived from glucose and fructose are present in foods as natural components. An overview of relatively commonly occurring oligosaccharides is shown in Table 4.14. Turanose, maltulose (**4-108**) and isomaltulose (4-**116**) are examples of reducing disaccharides (sucrose isomers).

Higher fructose oligomers linked by glycosidic bonds β-(2→6) are found in small amounts in foods. These sugars are mainly

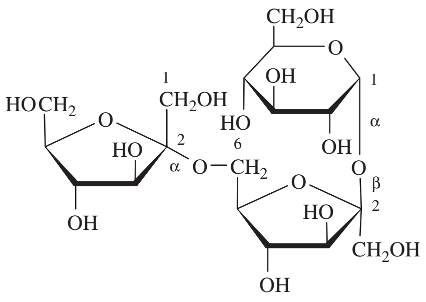
natural components of polysaccharides called fructosans (levans or phleines). The simplest substances of this type are trisaccharides 6-kestose (**4-119**), neokestose (**4-120**) and kelose (**4-121**). Trisaccharides 6-kestose and neokestose occur, for example, as minor components in molasses and invert sugar produced by acid hydrolysis of saccharose (inversion). The reaction by which these oligosaccharides are formed from lower saccharides is called **reversion**.



4-119, 6-kestose



4-120, neokestose



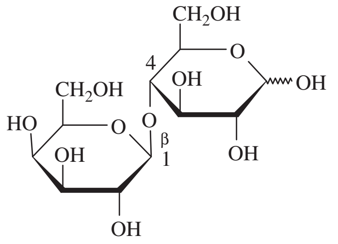
4-121, kelose

4.4.3 Galactooligosaccharides

4.4.3.1 Lactose

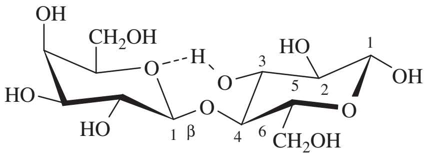
4.4.3.1.1 Structure and nomenclature

Lactose, *O*-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**4-122**), is a reducing disaccharide present in mammalian milk, and therefore lactose is also referred to as milk sugar. The most stable form is α-lactose monohydrate (α-anomer). Lactose crystallises in this form from aqueous solutions at temperatures up to 93.5 °C. Drying of lactose in vacuum at temperatures above 100 °C yields hygroscopic α-anhydride. Crystallisation from aqueous solutions at temperatures above 93.5 °C gives anhydrous β-lactose (β-anhydride). An amorphous hygroscopic mixture composed of α- and β-lactose forms during rapid drying of lactose solutions and also during drying of milk.



4-122, lactose

The mutarotation rate of lactose (as with other carbohydrates) depends on pH. The minimum rate of lactose mutarotation is in solutions of pH 4–5. In solutions of pH <2 and pH >7, the mutarotation rate increases significantly. Conformation of lactose in aqueous solutions (**4-123**) resembles the conformation of cellobiose.



4-123, conformation of lactose in aqueous solutions

4.4.3.1.2 Occurrence and production

Lactose is only synthesised in the mammary glands of mammals. Cows' milk contains 4–5% of lactose, human milk 5.5–7% of lactose. In addition to lactose, milk contains smaller amounts of D-glucose and a wide variety of free oligosaccharides. Lactose is also present naturally in all milk-containing products (e.g. milk chocolates and ice creams). Its content in the products prepared using homofermentative lactic acid bacteria (such as yoghurt, acidophilic milk and kefir) is lower than in fresh milk, generally about 1% or less.

Lactose is obtained by ultrafiltration of the whey of cows' milk or by crystallisation from whey concentrated to 55–65% of solids, which is called lactose syrup. Raw sugar is purified by recrystallisation.

4.4.3.1.3 Physiology and nutrition

Lactose is used as an energy source as it is hydrolysed by lactase in the small intestine. A large part of the human population (as well as of other mammals) produces lactase only in childhood. In adulthood, a number of individuals have the enzyme activity reduced or completely absent, and consumption of milk is then problematic. Lactase is produced by lactic acid bacteria that break

down lactose into lactic acid (L- or D-lactic acid or racemate). Fermented milk products, such as yoghurt and acidophilic milk, therefore, can also be consumed by people with lactase deficiency. The relative sweetening power of lactose is about 40% (20–60%) of the sweetening power of saccharose. Intake of lactose (as well as galactose intake) leads to a significant increase in blood glucose level. Lactose has relatively low cariogenic and laxative effects.

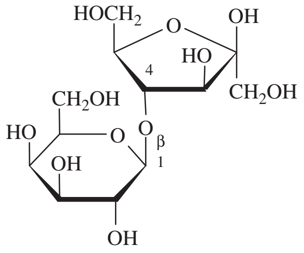
4.4.3.1.4 Use

Lactose is used as a sweet substance and also serves as a raw material for the production of oligosaccharides and some alditols:

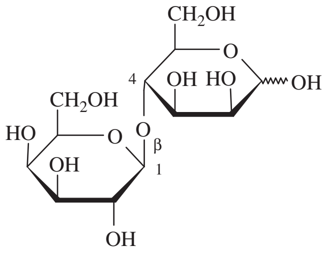
- lactulose
- lactosaccharose
- galactooligosaccharides
- lactitol
- galactose.

Lactulose

Lactulose (**4-124**) is obtained from lactose by isomerisation in alkaline solutions, where the main structure is β-pyranose followed by α-furanose and β-furanose. Epilactose is formed as a minor product (**4-125**). Lactulose is an indigestible disaccharide, which is somewhat sweeter than lactose (about 60% of sweetening power of sucrose) and has a weak laxative effect. It stimulates the growth of bifidogenic microflora.



4-124, lactulose



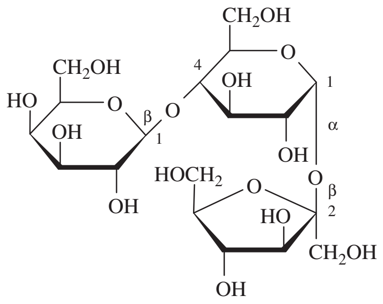
4-125, epilactose

Lactosaccharose

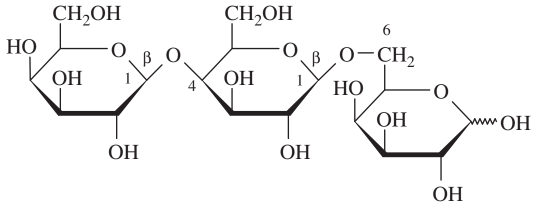
Lactosaccharose (**4-126**) is obtained from lactose and sucrose under the action of the transfructosylase activity of microbial β-fructofuranosidase.

Higher galactooligosaccharides

Galactooligosaccharides (also called transgalactooligosaccharides) are obtained from lactose using the galactosyltransferase activity of the enzyme β-galactosidase, which is reflected in solutions



4-126, lactosaccharose

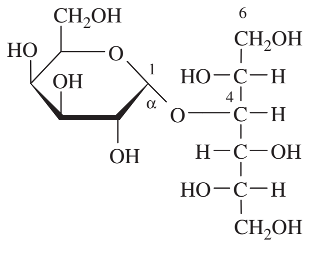


4-127, galactooligosaccharides

containing higher concentrations of lactose. These oligosaccharides contain 2–5 molecules of galactose (usually three molecules, so called galactotriose) joined by β-(1→6) bonds (**4-127**).

Lactitol

Lactitol, *O*-β-D-galactopyranosyl-(1→4)-D-glucitol (**4-128**) is an alcoholic sugar produced by hydrogenation of lactose at the reducing end. Hydrogenation of lactulose gives a mixture of lactitol and 4-*O*-β-D-galactopyranosyl-(1→4)-D-mannitol. Lactitol is currently used as a bulk sweetener in calorie-controlled foods. It has about 30–40% of sucrose sweetening power. As an indigestible and non-cariogenic compound with slight laxative properties, lactitol has no effects on blood glucose levels and insulin secretion.



4-128, lactitol

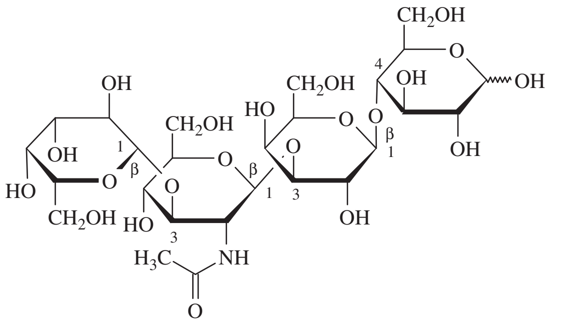
Galactose

Galactose can be obtained from vegetable raw materials containing galactans, but the most common source of galactose is lactose or protein-free whey. The process involves acid or enzymatic hydrolysis (by lactase) of galactans to an equimolar mixture of glucose and galactose. This mixture has about 40–60% of the sweetening power of saccharose. Galactose can be separated from

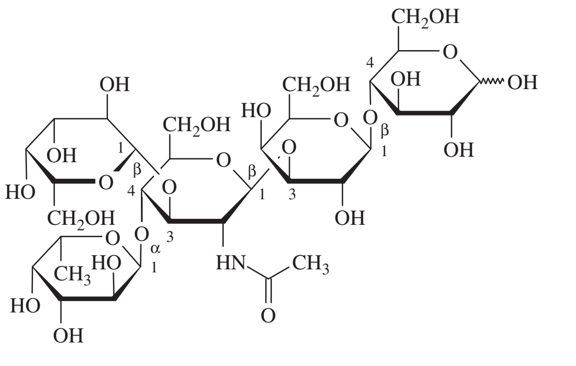
mixtures of galactose and glucose by selective fermentation of glucose to ethanol using various microorganisms or by glucose oxidase catalysed transformation of glucose to gluconic acid.

4.4.3.2 Other galactooligosaccharides

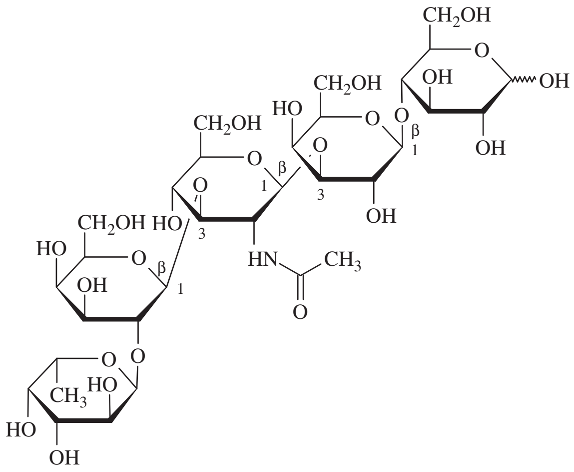
Breast milk and cows' milk contain, in addition to lactose, small amounts of mono- and oligosaccharides, such as D-glucose and D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, L-fucose and *N*-acetylneuraminic acid (*N*-acetyllactaminic acid) and a number of oligosaccharides derived from lactose. More than 130 oligosaccharides have been identified in breast milk. Some of these, lacto-*N*-tetraose (**4-129**), lacto-*N*-fucopentaose I (**4-130**) and lacto-*N*-fucopentaose II (**4-131**), are present in relatively large amounts (at around 1–2 g/l). The total content of higher oligosaccharides in human milk is 3–6 g/l (Table 4.15). These amino sugars of milk are a growth factor for microorganisms *Bifidobacterium bifidum*. They effectively inhibit the adhesion of pathogenic bacteria on cell walls, which is regarded as the initial phase of an infectious process. Therefore, these oligosaccharides play a significant role in non-immunological protection of infants. Similar oligosaccharides composed of D-galactose, *N*-acetyl-D-galactosamine and *N*-acetylneuraminic acids are components of κ-casein. An overview of other important galactooligosaccharides found in foods in given in Table 4.16.



4-129, lacto-*N*-tetraose



4-130, lacto-*N*-fucopentaose I



4-131, lacto-*N*-fucopentaose II

Soybeans, other legumes and also other foods of plant origin contain α-galactooligosaccharides raffinose (**4-132**), stachyose (**4-133**), verbascose (**4-134**), ajugose (**4-135**) and higher homologues that do not have trivial names (see Table 4.17). The highest member of the α-galactooligosaccharide series is nonasaccharide. These oligosaccharides can be considered derivatives of saccharose or melibiose. They are accompanied by a series of cyclitols and galactocyclitols (Table 4.9). Table 4.17 shows the content of main α-galactoligosaccharides in legumes.

α-Galactooligosaccharides are not digested by saccharases in the small intestine, but stimulate the growth of bifidobacteria and can be utilised by other bacteria of the colon that produce α-D-galactosidase and metabolise these sugars to form gases (carbon dioxide, methane and hydrogen). α-Galactooligosaccharides are considered the main cause of bloating (flatulence) in the consumption of legumes. Enzymatic hydrolysis by α-D-galactosidase can reduce levels of these oligosaccharides. Use of this enzyme, which catalyses the hydrolysis of raffinose, can improve the yield of saccharose from sugar beet.

So-called soy whey, which is the waste from the production of soy protein isolates and concentrates, can be used for the isolation of a mixture of α-galactooligosaccharides, which contains saccharose, glucose and fructose. In the form of syrup, this mixture can be used as a proteinaceous food additive.

4.4.4 Other oligosaccharides

Hydrolysis of waste wheat flour containing arabinoxylans, using the enzyme *endo*-1,4-xylanase, yields xylooligosaccharides containing D-xylose molecules linked by β-(1→4) bonds. The degree of polymerisation of these oligosaccharides is $n = 2–9$. Enzymatic synthesis using D-mannose has been used for the preparation of mannooligosaccharides containing 2–8 mannose molecules linked by α-(1→6) bonds. Both types of oligosaccharides can be used as

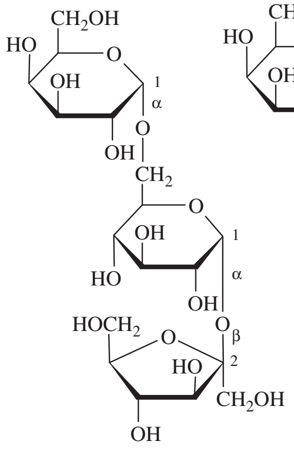
Table 4.15 Main oligosaccharides of breast milk and cows' milk.

Oligosaccharide	Abbreviated notation[a]	Content (g/l)	
		Breast milk	Cows' milk
Lactose	β-D-Galp-(1→4)-D-Glcp	55–70	40–50
Lacto-*N*-tetraose	β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	0.5–1.5	traces
Lacto-*N*-fucopentaose I	α-L-Fucp-(1→2)-β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	1.0–1.5	–
Lacto-*N*-fucopentaose II	β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	0.5–1.0	–
	4		
	↑		
	1		
	α-L-Fucp		
Sialyl-α-(2→6)-lactose	α-NeupAc-(2→6)-β-D-Galp-(1→4)-D-Glcp	0.3–0.5	0.03–0.06[b]
Sialyllactotetraose a	α-NeupAc-(2→3)-β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	0.03–0.2	traces
Sialyllactotetraose c	α-NeupAc-(2→6)-β-D-Galp-(1→4)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	0.1–0.6	traces
Disialyllacto-*N*-tetraose	α-NeupAc-(2→3)-β-D-Galp-(1→3)-β-D-GlcpNAc-(1→3)-β-D-Galp-(1→4)-D-Glcp	0.2–0.6	traces
	6		
	↑		
	2		
	α-NeupAc		

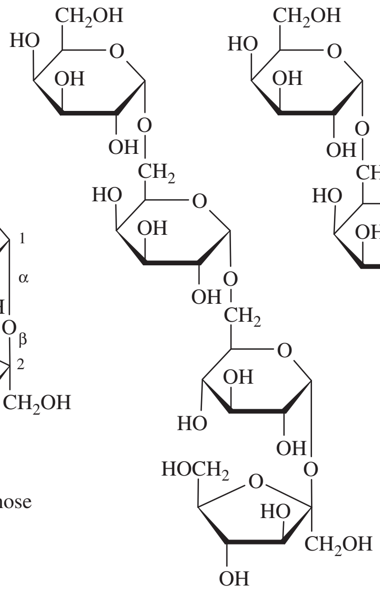
[a] Fuc = fucose, GlcNAc = *N*-acetylglucosamine, GalNAc = *N*-acetylgalactosamine, NeuAc = *N*-acetylneuraminic acid.
[b] Together with sialyl-α-(2→3)-lactose.

Table 4.16 Overview of important galactooligosaccharides.

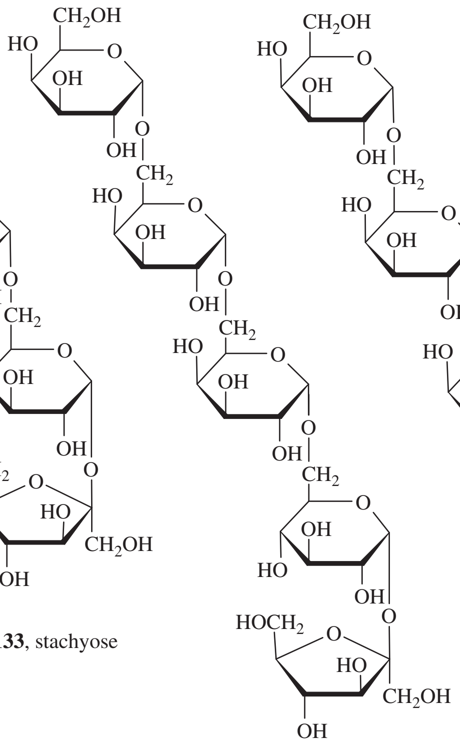
Trivial name	Abbreviated notation	Occurrence
Disaccharides		
Melibiose	α-D-Galp-(1→6)-D-Glcp	Cocoa beans, grapes
Lactose	β-D-Galp-(1→4)-D-Glcp	Milk, diary products
Lactulose	β-D-Galp-(1→4)-D-Fruf	Milk, diary products
Epilactose	β-D-Galp-(1→4)-D-Manp	Milk, diary products
Solabiose	β-D-Glcp-(1→3)-D-Galp	Glycosides
Higher oligosaccharides		
Manninotriose	α-D-Galp-(1→6)-α-D-Galp-(1→6)-D-Glcp	Legumes
Raffinose	α-D-Galp-(1→6)-α-D-Glcp-(1↔2)-β-D-Fruf	Sugar beet, legumes, grapes
Umbelliferose (isoraffinose)	α-D-Galp-(1→2)-α-D-Glcp-(1↔2)-β-D-Fruf	Root vegetables
Planteose	α-D-Galp-(1→6)-β-D-Fruf-(2↔1)-α-D-Glcp	Cocoa beans, tobacco, spices
Stachyose	α-D-Galp-(1→6)-α-D-Galp-(1→6)-α-D-Glcp-(1↔2)-β-D-Fruf	Legumes, grapes, artichokes
Verbascose	α-D-Galp-(1→6)-[α-D-Galp-(1→6)]$_2$-α-D-Glcp-(1↔2)-β-D-Fruf	Legumes
Ajugose	α-D-Galp-(1→6)-[α-D-Galp-(1→6)]$_3$-α-D-Glcp-(1↔2)-β-D-Fruf	Legumes



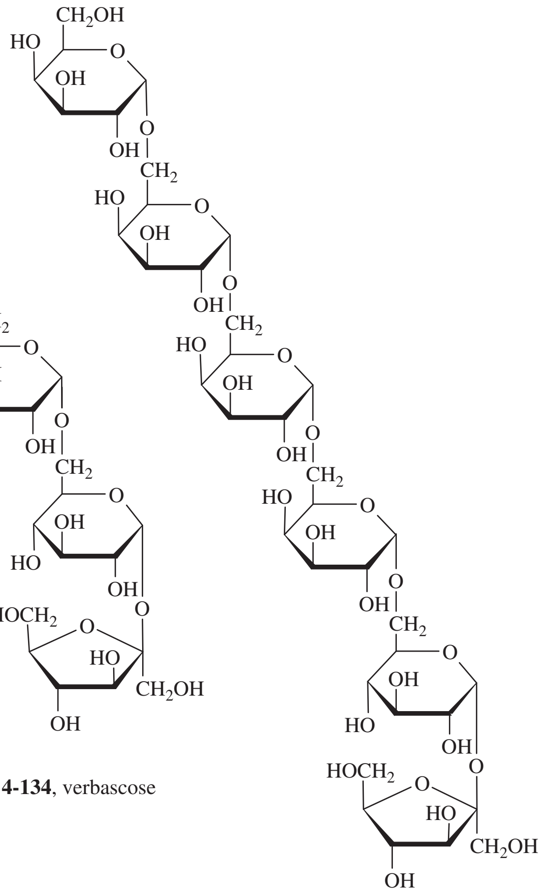
4-132, raffinose



4-133, stachyose



4-134, verbascose



4-135, ajugose

Table 4.17 Contents of major oligosaccharides in legume seeds (% of dry matter).

Legumes	Latin name	Saccharose	Raffinose	Stachyose	Verbascose
Common bean	*Phaseolus vulgaris*	2.2-4.9	0.3-1.1	3.5-5.6	0.1-0.3
Vigna mungo	*Vigna mungo*	1.3	0.3	1.7	2.8
Pea	*Pisum sativum*	2.3-3.5	0.6-1.0	1.9-2.7	2.5-3.1
Lentil	*Lens culinaris*	1.3-2.0	0.3-0.5	1.9-3.1	1.2-1.4
Soybean	*Glycine max*	2.8-7.7	0.2-1.8	0.02-4.8	0.1-1.8
Chickpea	*Cicer arietinum*	2.0-3.5	0.7-0.9	1.5-2.4	0.0

food additives because they possess similar physiological effects as other unavailable oligosaccharides.

4.5 Polysaccharides

4.5.1 Structure and nomenclature

Polysaccharides or glycans consist of more than ten monosaccharide units and can contain up to several thousands, hundreds of thousands or even around a million structural units linked to each other by glycosidic bonds.[3]

Polysaccharides are composed either of identical monomers (excluding terminal units) or more often of molecules of two or more different monosaccharides, or they contain monosaccharide

[3]The name polyglycose is not synonymous with the name glycan since polyglycose can be a polymer in which monosaccharides are also linked by means other than glycosidic bonds.

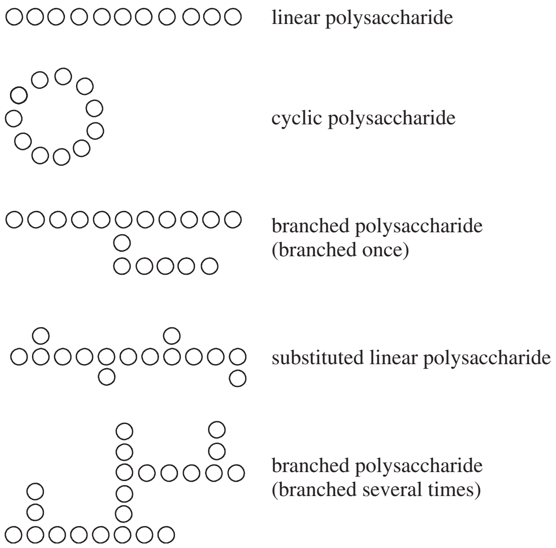


Figure 4.6 Structure of polysaccharide chains.

derivatives such as glycuronic acids, their esters and deoxysugars. It is therefore possible to distinguish:

- homopolysaccharides (homoglycans)
- heteropolysaccharides (heteroglycans).

Homoglycans are, for example, components of starch, amylose and amylopectin, glycogen and of cellulose, which are composed exclusively of D-glucose molecules. Most of the other polysaccharides are heteroglycans. Polysaccharide (and also oligosaccharide) chains (Figure 4.6) can be:

- linear (e.g. amylose and cellulose)
- cyclic (e.g. higher cyclodextrins).

Linear chains can be:

- unbranched (as in amylose and cellulose)
- branched (as in amylopectin).

The branched chains can be branched only once as in dextran, substituted as in guar gum or can be branched several times as in amylopectin.

Names of homoglycans are derived from the monosaccharide name by substitution of the ending -ose by the ending -an. Some older trivial names have changed, and coincide with today's terminology (e.g. caragenin or carrageenin or carrageenan); others are still in use (dextrin, pectin and inulin, and also starch and cellulose).

The building blocks of homopolysaccharides are mostly pentoses, hexoses and glycuronic (alduronic) acids. Their respective polymers are called:

- **pentosans**
- **hexosans**
- **glycuronans** (formerly also **polyuronides**).

The most common pentose bound in pentosans is D-xylose present in **xylans**. The most common building blocks of hexosans are D-glucose, D-mannose, D-galactose and D-fructose. Homopolysaccharides, termed **glucans**, are composed entirely of glucose units (amylose, amylopectin and cellulose). If glycosidic bonds link exclusively the α-anomers of a monomer, the glucans are called α-glucans (amylose). Cellulose is β-glucan or, more precisely, **β-(1→4)-glucan**. **Fructans** are composed exclusively from fructose units, **mannans** of mannose units and **galactans** of galactose units. Common components of many polysaccharides are alduronic acids, such as D-galacturonic acid in pectin and D-mannuronic and L-guluronic acids in alginates.

Names of heteroglycans having the main chain composed of a single type of monosaccharide, ends with the name of the homopolysaccharide forming the main chain. Other sugar residues present in side chains are shown in alphabetical order before the basic name. For example, pentosans include **arabinoxylans**, whose main chain consists of D-xylose units and the side chain contains L-arabinose. Sugars D-xylose and D-glucose occur in **xyloglucans**. Hexoses D-glucose, D-fructose, D-mannose and D-galactose are common constituents of heteropolysaccharides known as **glucofructans** (inulins and phleins) **glucomannans** (konjac gum) and **galactomannans** (guar gum). If the main polysaccharide chain is not homopolymeric, all monosaccharide residues contained in the chain are shown in alphabetical order. Examples of such heteropolysaccharides are **arabinoglucuronoxylans**. An overview of the main building blocks of major polysaccharides is presented in Table 4.18.

Polysaccharides mostly have the reducing monosaccharide residue at one end of the chain, but some may have the non-reducing residues on both ends. Linear homopolysaccharide amylase has monosaccharide non-reducing residue at the beginning of the chain, and the end of the chain unit consists of a reducing unit with a hemiacetal hydroxyl group. Branched polysaccharides, such as amylopectin, have one reducing unit and $n + 1$ non-reducing units at the beginning of each of the n molecule chain branches.

The **primary structure** of a polysaccharide (monosaccharide sequences) is regular in homoglycans (such as amylose and cellulose) and in some heteroglycans (arabinoxylans). The individual monosaccharides are also regularly alternating in a number of heteroglycans (carrageenan), or the regular structure is disrupted and the order of monomers varies (e.g. in pectin) in certain chain sections.

The type of monosaccharide units, their conformation and type of linkages affect the conformation of macromolecules, the so-called glycan **secondary structure**. Linear macromolecules, such as cellulose, are often stabilised by hydrogen bonds between hydroxyl groups of one molecule of glucose and oxygen atoms of the second pyranose ring. Another type of conformations are known as egg

Table 4.18 Main building monosaccharide units of important polysaccharides.

Monosaccharide	Abbreviated notation	Polysaccharides
α-L-Arabinofuranose	α-L-Ara*f*	Plant gums (arabic, larch, ghatti), pectins, arabinoxylans
β-L-Arabinopyranose	β-L-Ara*p*	Plant gums (arabic, larch, ghatti), pectins, arabinoxylans
α-D-Xylopyranose	α-D-Xyl*p*	Arabinoxylans, xyloglucans
β-D-Xylopyranose	β-D-Xyl*p*	Tragacanth
α-L-Rhamnopyranose	α-L-Rha*p*	Pectins, plant gums (arabic, karaya), okra, gellan gum
α-L-Fucopyranose	α-L-Fuc*p*	Xyloglucans, pectins, tragacanth
α-D-Glucopyranose	α-D-Glc*p*	Starches, modified starches and their derivatives, starch hydrolysates, glycogen, dextran, elsin, pullulan
β-D-Glucopyranose	β-D-Glc*p*	Cellulose, modified celluloses, xyloglucans, glucomannans, β-glucans with mixed linkages, xanthan, gellan gum, curdlan, scleroglucan
β-D-Glucopyranuronic acid	β-D-Glc*p*A	Pectins, plant gums (arabic, karaya, ghatti)
α-D-Mannopyranose	α-D-Man*p*	Xanthan, plant gums (ghatti)
β-D-Mannopyranose	β-D-Man*p*	Galactomannans, glucomannans, xanthan
β-D-Mannopyranuronic acid	β-D-Man*p*A	Alginates
α-L-Gulopyranuronic acid	α-L-Gul*p*A	Alginates
α-D-Galactopyranose	α-D-Gal*p*	Galactomannans, pectins, plant gums (arabic, karaya), okra
β-D-Galactopyranose	β-D-Gal*p*	Xyloglucans, pectins, plant gums (arabic, larch, ghatti, tragacanth), agar, carrageenans
α-D-Galactopyranuronic acid	α-D-Gal*p*A	Pectin, plant gums (tragacanth), okra
3,6-Anhydro-α-D-galactopyranose	α-D-Gal*p*3,6An	Carrageenans
3,6-Anhydro-α-L-galactopyranose	α-L-Gal*p*3,6An	Agar
β-D-Fructofuranose	β-D-Fru*f*	Glucofructans and fructans (inulins and phleins)

box conformations, which have hydrogen bonds between hydroxyl groups of monomers and calcium cations (in pectins), or ionic bonds between dissociated carboxyl groups and calcium ions (e.g. alginates). For some polysaccharides, helical conformations are typical (e.g. in carrageenans).

The combinations of secondary structures form **tertiary structures**, such as crystalline microfibrils in cellulose or double and triple helices in κ-carrageenan.

Polysaccharides are polydisperse substances, as they occur as mixtures of polymers with different degrees of polymerisation, and thus have an average molecular weight.

4.5.2 Classification

Polysaccharides are commonly divided according to their origin. Natural **polysaccharides of plants** have the greatest importance in human nutrition, while **polysaccharides of animals** and other natural polysaccharides have little or no importance. Many polysaccharides of plants (guar gum or locust bean gum), seaweed (such as agar, carrageenans and alginates) and microorganisms (e.g. xanthan gum) are becoming part of many foods as additives in natural or modified forms (modified starches and celluloses and modified chitin called chitosan).

According to the basic functions that are executed in the tissues of animals, tissues and cells of plants, algae, higher fungi and microorganisms, polysaccharides are divided into:

- **storage** or **reserve polysaccharides**
- **structural polysaccharides**
- **polysaccharides with other functions**.

The storage polysaccharide in animals is glycogen. Structural functions are carried out by chitin, forming the exoskeletons of crustaceans, molluscs and insects, and mucopolysaccharides occurring in proteoglycans of connective tissues.

Plant storage polysaccharides in seeds, tubers, rhizomes, bulbs and roots are:

- **starches** formerly also known as amyloids (e.g. in cereals, legumes and potato tubers)

- **non-starch polysaccharides**, which include glucofructans and fructans (chicory root and cereal seeds), galactomannans that are known as seed gums (storage polysaccharides of some legumes, such as guar gum and locust bean gum), glucomannans (konjak tubers) and xyloglucans (e.g. rape and tamarind seeds).

Functioning as building materials in the walls of plant cells are:

- **cellulose**

- **non-cellulose polysaccharides** associated with cellulose.

The non-cellulose polysaccharides include:

- **hemicelluloses** (xyloglucans in fruits, most vegetables, root crops and legumes, arabinoxylans and β-glucans in cereals and galactomannans in some legumes)

- **pectins** (in fruits and vegetables).

The polymer of phenylpropanoid units is **lignin**, which is not composed of saccharide units, but is associated with cellulose as well as with structural non-cellulose polysaccharides, is also a structural material of plant cell walls. Lignin is accompanied by other phenolic compounds (tannins), proteins and polymers of lipids.

Other plant polysaccharides seem to have different functions related to water management and protection of damaged tissues. These polysaccharides include:

- **plant exudates** or **gums** of some plants (such as gum arabic and tragacanth)

- **mucilages** (e.g. okra).

Some structural polysaccharides from algae are used for consumption as food additives, especially agar, carrageenans and alginates. Some extracellular polysaccharides of microorganisms also have significance as food additives (such as xanthan gum). They have functions other than structural and reserve. Some extracellular and structural polysaccharides of microorganisms and higher fungi, such as β-glucans with a different structure to that of β-glucans of cereals, have found use as immunomodulators and anticarcinogenic substances.

4.5.3 Natural occurrence in foods

Pectin is the dominant polysaccharide in fruits, which also contain (in smaller amounts) some other polysaccharides, cellulose and hemicelluloses (the predominant components are xyloglucans) and lignin. Starch is only found in immature fruits (e.g. about 2.5% in immature apples) and its content decreases during ripening, and is virtually absent in ripe fruits. The exceptions are bananas, which even at full maturity contain at least 3% starch by weight and about 1% glucofructans (fructans).

Table 4.19 Content of main polysaccharides and lignin in wheat flour.

Polymer	Content (%)	Polymer	Content (%)
Starch	60–80	β-Glucans	0.5–2
Nonstarch polysaccharides	3–11	Xyloglucans	0.2–0.4
Cellulose	0.2–3	Pectins	0.3–0.5
Hemicelluloses	2–7	Glucofructans (fructans)	1–4
Arabinoxylans	1–3	***Lignin***	0.7–12

Starch is the predominant polysaccharide of root vegetables and root crops (potatoes). Unlike fruits, its content increases during ripening and a higher content of starch is found in over-ripe vegetables. In dicotyledonous (dicot) vegetable plants of the aster family (Asteraceae), such as black root, artichokes (grown mainly for the inflorescences, which are consumed fresh or preserved), Jerusalem artichokes (tubers) and in bulbs of monocotyledonous (monocot) plants of the lily family (Liliaceae), such as garlic and onion, the main reserve carbohydrates are glucofructans (fructans). Cellulose, hemicelluloses (predominantly xyloglucans), pectins and lignin are important components of most vegetables.

Starch is also the major polysaccharide in all cereals. Of the non-starch polysaccharides, the predominant ones are hemicelluloses, whose main components in wheat and rye are arabinoxylans and in oat and barley so called β-glucans. Also found in significant quantities are glucofructans (fructans), xyloglucans, cellulose and lignin, which is mainly present in the bran. The main polysaccharides contents of wheat flour are given in Table 4.19. The content of polysaccharides varies widely, particularly since it depends on the degree of milling (known as flour extraction rate; the highly straight run flours are higher in non-starch polysaccharides) and other factors. Endosperm represents about 83% of grain by weight, bran 15% and germ 2%. The starch content in the bran is about 15%, but the cellulose content is about 35% and the content of hemicelluloses reaches 40–50%.

4.5.4 Physiology and nutrition

From the nutritional point of view the following are recognised:

- **utilisable polysaccharides**

- **non-utilisable polysaccharides** (formerly known as ballast polysaccharides), since the enzyme machinery for their digestion in humans and other monogastric animals is lacking and they are not cleaved by saliva, the pancreas or small intestine saccharases.

Table 4.20 Fibre composition of fruits, vegetables and cereals (in %).

Fibre source	Noncellulose polysaccharides		Cellulose		Lignin	
	Range	Average	Range	Average	Range	Average
Fruits	46–78	62.9	9–33	19.7	1–38	17.4
Vegetables	52–76	65.6	23–42	31.5	0–13	3.0
Cereals	71–82	75.7	12–22	17.4	0–15	6.7

Utilisable plant polysaccharides are starches (the main source of energy) and animal glycogen. Non-cellulose polysaccharides include hemicelluloses and pectin, as well as polysaccharides used as food additives (seaweed polysaccharides, microbial polysaccharides, vegetable gums and mucilages, modified polysaccharides) and lignin and chitin of animal polysaccharides (and modified chitin, or chitosan). Some non-cellulose polysaccharides, such as pectin, can be relatively easily utilised (see Table 4.22 later), but these substances are known by the widespread and accepted term **fibre** or **dietary fibre**, although the name is misleading, inaccurate and poorly definable. Some older definitions included plant polysaccharides (except starch) and lignin under the term dietary fibre, which are materials resistant to hydrolysis by human digestive juices. Other definitions included all undigested and unabsorbed food components, including undigested proteins, minerals and phytates, and possibly also endogenously excreted mucopolysaccharides. The EU definition of dietary fibre describes carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed;
- edible carbohydrate polymers that have been obtained from food raw material by physical, enzymatic, or chemical means, which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
- edible synthetic carbohydrate polymers, which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.

The current definition of dietary fibre is based on the chemical composition of polysaccharides and includes all non-utilisable carbohydrates, including polysaccharides and oligosaccharides used as food additives (e.g. plant gums and mucilages, algal and microbial polysaccharides and modified starches, celluloses and chitosan). Regarding lignin, it is stated that the carbohydrate polymers of plant origin that meet the definition of fibre may be closely associated in the plant with lignin or other non-carbohydrate components such as phenolic compounds, waxes, saponins, phytates, cutin and phytosterols. The definitions do not take into account the fact that fibre is not a static concept, as it does not pass through the digestive system completely intact. A substantial component of dietary fibre (due to the amount consumed) is a certain percentage of not-completely-degraded starch known as **resistant starch**. The gross composition of the dietary fibre of fruits, vegetables and cereals is presented in Table 4.20.

According to the solubility in water, the following two categories are recognised:

- **soluble dietary fibre**
- **insoluble dietary fibre**.

Soluble dietary fibre includes a certain proportion of hemicelluloses. For example, about one third of cereal structural arabinoxylans are soluble. Also, one quarter to about one half of barley β-glucans are soluble, and even a certain proportion of glucomannans and galactomannans of legumes are soluble. Other soluble polysaccharides are pectins, plant mucilages, seaweed polysaccharides, modified starches and modified celluloses (Table 4.21).

The main components of insoluble fibre include cellulose, a certain proportion of hemicelluloses and lignin. Higher lignin content is found in the bran and seeds of consumed fruits, such as garden strawberries, raspberries and currants. Insoluble fibre increases the volume of food, reduces the time of its passage through the digestive tract and increases peristalsis. Soluble fibre increases the viscosity of the contents of the stomach and intestines, slows down the mixing of their content and limits the access of pancreatic amylase and lipase to substrates. As a result, the nutrient absorption by the intestinal wall slows down; slower passage of the intestinal contents reduces the diffusion of bound nutrients and minerals (especially calcium, iron, copper and zinc ions), and thus their availability is modified. Part of the bound ions are released during fermentation in the colon. Accompanying tannins also partially inhibit digestive enzymes.

Dietary fibre is a beneficial material for constipation, gastric and duodenal ulcers, haemorrhoids, rectum and colon (colorectal) cancer, commonly known as bowel cancer, and other diseases. Eating foods high in fibre is recommended for the modulation of glucose levels in blood serum and in some forms of diabetes (especially type 2 diabetes). This also has the effect of reducing serum cholesterol level, and can thus help to prevent cardiovascular diseases. The effect is explained by the reduced absorption of cholesterol from viscous foods and by cholesterol binding to fibre,

Table 4.21 Proportion of soluble and insoluble dietary fibre in selected foods.

	Dietary fibre (% of dry matter)				Dietary fibre (% of dry matter)		
Food	**Soluble**	**Insoluble**	**Total**	**Food**	**Soluble**	**Insoluble**	**Total**
Fruits				***Legumes***			
Apples	5.6–5.8	7.2–7.5	12.8–13.3	Beans	7.2–12.4	9.1–9.6	16.8–21.5
Peaches	4.1–7.1	3.4–6.4	7.5–13.5	***Potatoes***			
Strawberries	5.1–7.7	6.8–10.6	11.9–18.3	Raw	2.8–3.5	2.4–3.2	5.2–6.7
Oranges	6.5–9.8	3.9–5.2	10.4–15.0	Boiled	4.8	2.6	2.2
Vegetables				***Cereals***			
Carrot	4.4–14.9	10.4–11.1	14.8–26.0	Wheat flour (white)	2.0	1.2	3.2
Cabbage	13.5–16.6	4.2–20.8	27.6–37.4	Wheat flour (whole)	2.6	7.7	10.3
Tomatoes	0.8–3.5	3.2–12.8	6.7–13.6	Wheat bread	1.6–2.7	1.1–2.9	2.7–5.6
Peas	5.9	15.0	20.9	Rye bread	6.7	6.6	13.3

which results in increased excretion of cholesterol in the faeces. Bile acids are also bound to dietary fibre and excreted. The result is a decrease of bile acid reserves in the liver. This deficit of bile acids has to be compensated by biosynthesis from cholesterol, which reduces the cholesterol concentration in the blood plasma. Cholesterol synthesis in the liver is also inhibited by lower fatty acids being formed during fibre fermentation by intestinal microflora.

Soluble fibre is partially hydrolysed by digestive enzymes already in the small intestine. Insoluble fibre is not hydrolysed in the small intestine, and along with soluble fibre is partly metabolised only by colon and appendix microorganisms (e.g. xanthan gum is not metabolised at all). Colon and appendix microorganisms assimilate on average 70% of dietary fibre polysaccharides (Table 4.22). The final products are gases (carbon dioxide and hydrogen, often methane) and lower fatty acids (acetic, propionic and butyric acids). The amount of energy obtained in the form of these acids is around 3 kJ/g carbohydrate (compared with about 17 kJ/g obtained from starch). It is estimated that the consumption of 20–30 g of dietary fibre (the Recommended Daily Allowance of dietary fibre is >25 g per day in Europe) contributes to the total energy intake in the range of 0.5 to 1%; the rest is covered by the main nutrients. The ratio of insoluble and soluble fibre in the diet should be 3 : 1.

Table 4.22 Utilisation of soluble fibre components.

Polysaccharide	Utilisation (%)	Polysaccharide	Utilisation (%)
Hemicelluloses	19–85	Karaja gum	5
Pectins	65–97	Agar	21–28
Guar gum	76	Carrageenans	9–16
Locust gum	<15	Alginates	0–78
Gum arabic	71	Dextran	78–90

4.5.5 Properties and use

Polysaccharides in foods contribute to the formation of texture and also affect other organoleptic properties. Soluble polysaccharides are used in many food industry sectors and other fields as fillers, thickeners, to increase the viscosity of products, act as stabilisers of dispersions and some are thickening agents and gelling substances.

The importance of polysaccharides has increased with the development of new technologies and of products with reduced fat and saccharose contents. Previously the market was dominated by native starch, but its consumption is declining and consumption of modified starches is significantly increasing. Important substances are modified celluloses, plant gums and polysaccharides of seaweeds and microorganisms. At the forefront of world consumption of non-starch polysaccharides are plant seed gums (guar gum and locust gum), followed by carrageenan, agar, gum arabic, pectins, alginates, modified celluloses (carboxymethyl cellulose) and xanthan gum. There have also been major increases in the sale of food supplements containing natural soluble fibre, which occurs mainly in the form of arabinoxylans (such as psyllium dietary fibre supplements),[4] modified fructans and chitosan.

4.5.6 Plant polysaccharides

4.5.6.1 Starch

Starch is the main plant storage polysaccharide, which serves as a prompt supply of energy. Unlike structural polysaccharides, which are part of the plant cell walls, starch is found in organelles

[4] *Psyllium* is the common name used for several members of plantains that belong to the genus *Plantago* (Plantaginaceae). For example, sand plantain (*P. psyllium*) is grown for seeds known as black, French or Spanish psyllium.

Table 4.23 Basic characteristics of starch granules.[a]

Source	Diameter (μm)	Mean value (μm)	Source	Diameter (μm)	Mean value (μm)
Wheat	4-6; 15-25	15	Potatoes	38-50	33
Barley	3-5; 19-25	15	Cassava	6-36	20
Rice	3-9; 15-30	5	Amaranth	1.07-1.10	1.1

[a]Cereal starches have bimodal distribution of granules. Large granules (A type granules) have a lens shape and diameter of about 20 μm, small granules (B type granules) are spherical particles with a diameter of about 5 μm.

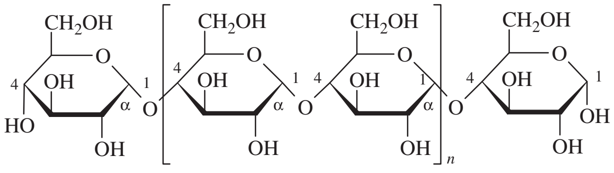
of cytoplasm called plastids, where its biosynthesis takes place. Chloroplasts of the specialised photosynthetic tissues produce so-called **transient starch** during the day, which serves as a temporary sugar reserve. Storing of glucose obtained by photosynthesis in the form of starch strongly reduces high intracellular osmotic pressures, to which cells would otherwise be exposed. All transient starch granules synthesised during the day undergo nocturnal breakdown and serve as a major source for saccharose synthesis at night that is needed for metabolism in the whole plant. Saccharose is then transported to the storage organs of plants, such as seeds, fruits, tubers and roots, where the specialised leucoplasts, known as amyloplasts,[5] synthesise and store the so-called **reserve starch**. This starch is stored in insoluble micelles called **starch granules** or starch grains that have species-specific shapes (e.g. round and oval) and dimensions (Table 4.23).

4.5.6.1.1 Structure

The majority of native starches are mixtures of **amylose** and **amylopectin**, two homopolysaccharides composed of α-D-glucopyranose molecules in $^4C_1$ conformation. Amylose and amylopectin usually occur in a weight ratio of 1 : 3. In some varieties of cereals (maize, barley and rice) and other plants (potatoes), either amylose (starches of high amylose content or amylostarches) or amylopectin (starches high in amylopectin or waxy starches) predominate.

Amylose

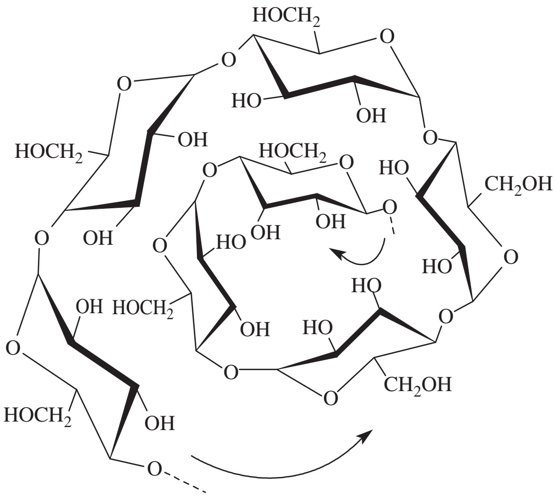
Amylose is a linear α-D-(1→4)-glucan (**4-136**), and, therefore, is actually a polymer of the disaccharide maltose. To a limited extent, branching occurs at about ten sites of the molecule. Amylose is partially esterified with phosphoric acid (wheat starch contains 0.055% and potato starch 0.07–0.09% of phosphorus) and in cereal starches forms complexes with lipids. An amylose molecule has one reducing monosaccharide residue.



α-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)$_n$-α-D-Glc*p*

4-136, amylose

Owing to the prevailing bonds 1 (axial) → 4 (equatorial), the amylose molecule can be randomly coiled in water and in neutral solutions, but generally tends to wind up into a rather stiff left-handed single helix or forms even stiffer parallel left-handed double helical junction zones (**4-137**). Single helical amylose has hydrogen-bonded O-2 and O-6 atoms. In alkaline solutions, globular structures dominate. As with each polysaccharide, amylose is a mixture of polymers with different degrees of polymerisation. The average content of glucose units is 1000–2000 (in cereal starches), but also up to around 4500 (in potato starch). The molecular weight of amylose ranges between 180 and1000 kDa.



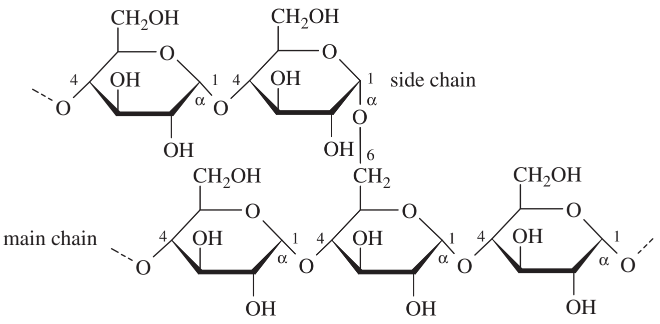
4-137, helical section of amylose molecule

Amylopectin

An amylopectin molecule (**4-138**) consists of chains of D-glucose units linked by α-(1→4) linkages (maltose polymer), which is branched after 10 to 100 (average of 25) units by non-random α-(1→6) side chains (building unit is isomaltose). Occasionally there may also be an α-(1→3) bond (building unit of this biose is laminaribiose). One of about 400 glucose residues is esterified with phosphoric acid.

The degree of polymerisation is 50 000–1 000 000, thus the molecular weight varies between 10 and 200 MDa. Amylopectin macromolecule is a many times branched structure consisting of three types of chains: outer chain A, inner chain B and main chain C. About 5% of glucose molecules form the branch points. The inner and outer short side chains are called S chains and internal

[5]Specialised amyloplasts that are heavier than the cytoplasm are statolites, located in cells at the tips of roots. They are used to register the direction of Earth's gravity by plants.



$\cdots\!\rightarrow$4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*
1
↓
6
$\cdots\!\rightarrow$4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-((1→4)-α-D-Glc*p*-((1→$\cdots$

4-138, amylopectin

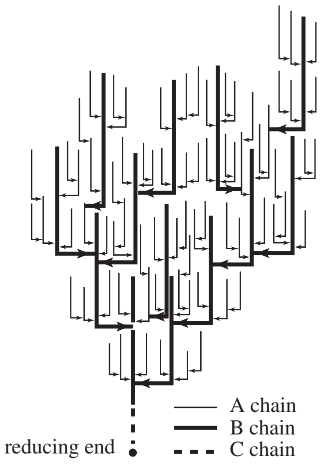


Figure 4.7 Schematic structure of the amylopectin molecule. Robin, Mercier, Charbonniere, Guilbot 1974, fig 11. Reproduced by permission of AACC International.

long chains are L chains in modern terminology. The amylopectin molecule has one reducing end in the main chain (Figure 4.7).

Starch granules

The ultrastructure of starch granules varies depending on the plant source, but they have a common general model (Figure 4.8). The amylopectin molecules are oriented radially (from the centre to the edge) in the starch granule. The non-reducing amylopectin ends are situated outside the granules and form their surface. In areas of non-reducing ends and in the central parts of the chains (corresponding to the length of about 15 glucose units) antiparallel double helices with an ordered (crystalline) three-dimensional structure are formed. The presence of amylose in starch tends to reduce the crystallinity of the amylopectin, and influences the ease of water penetration into the granules. In

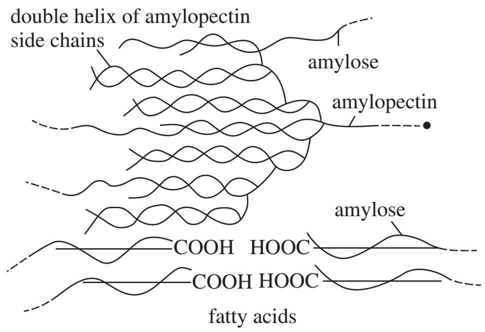


Figure 4.8 Arrangement of starch grain components. Stephen 1995, fig 2.3. Reproduced by permission of Taylor & Francis – Marcel Dekker.

areas of chain branching, amylopectin and the accompanying amylose have an unordered amorphous structure. Crystalline and amorphous regions alternate regularly. Depending on the degree of crystallinity of the granules (related to the structure and chirality of double helices of amylopectin side chains in crystalline regions of granules), four polymorphic forms of starch have been identified. They are labelled A, B, C and V. The most stable form, A, is found in cereal starches with the exception of starches with high content of amylose. A double spiral creates a central channel that is filled by another double helix and in the space between the double helices is bound water (tighter than in form B). The least stable form is form B, where the central channel double helix is only filled by water molecules. This form occurs in starches of root vegetables, potatoes and starches of cereals with high amylose content (>40%). Form C is present in legumes (it can be a combination of forms A and B). Retrogradation in gelatinised starches with amorphous structure first forms the less stable form B that is subsequently transformed into form C, which is transformed into the most stable form A. Form V occurs in gelatinised starch containing lipids, in which amylose interacts with fatty acids.

The amylopectin molecules are associated with amylose molecules that form in certain parts of the molecule of left-handed helices, oriented with non-reducing ends to the surface of the granules. They are located mainly in the amorphous zones, together with radially oriented lipid molecules whose fatty acids are immersed in helical parts of amylose molecules. These interactions create non-stoichiometric complexes called inclusion compounds. An integral part of the granules are also small amounts of proteins with relative molecular weights of 5–97 kDa. For example, the hardness of the endosperm of cereals is attributed to the protein friabilin (15 kDa). Proteins are found mainly in the surface layers of granules (Table 4.24).

4.5.6.1.2 Occurrence and production

The starch content and its composition (amylose content) in some foods is given in Table 4.25, amylopectin content is the difference to make up the 100%. The main sources of starch in foods and industrial sources of starch are potatoes (*Solanum tuberosum*, Solanaceae) and cereals, especially wheat (*Triticum aestivum*, Poaceae), rye (*Secale cereale*), barley (*Hordeum vulgare*), oats

Table 4.24 Content of lipids and proteins in starches.

	Content (%)			Content (%)	
Starch	Lipids	Proteins	Starch	Lipids	Proteins
Wheat	0.38–0.72[a]	0.3	Peas	0.1	0.7
Maize	0.02–1.09	0.3	Potatoes	0.05	0.06
Beans	0.1	0.9	Cassava	0.1	0.1

[a]Lipids in starch form 25% of total flour lipids; the main components are lysophospholipids.

Table 4.25 Content of starch and its composition in important sources.

Source	Starch (%)	Amylose (%)
Amaranth	48–69	0–22
Barley[a]	52–62	38–44
Beans[b]	46–54	24–33
Cassava	28–35	17–19
Maize	65–75	24–26
Oats	40–56	25–29
Potatoes[c]	17–24	20–23
Rice	70–80	8–37
Rye	52–57	24–30
Sweet potatoes	10–30	19–25
Wheat	59–72	24–29

[a]Waxy barley contains 2–8% amylose, waxy maize about 1%, and barley amylostarch and maize amylostarch 60–70%.
[b]Lentils and ripe seeds of peas have similar levels of starch and amylose as beans. Green peas have about 4% starch, soybeans contain less than 1% starch.
[c]Developed potato varieties have the starch content at the upper limit of that range.

(*Avena sativa*), maize (*Zea mays*) and rice (*Oryza sativa*) and also new pseudocereals, such as amaranth (*Amaranthus hypochondriacus*). Important sources of starch are also ripe seeds of legumes, such as pea (*Pisum sativum*, Fabaceae), various types of beans (*Phaseolus* spp.) and lentils (*Lens culinaris*). The starch content of cereal grains ranges from 40 to 90% of dry matter, legumes contain 30–70% of starch and plant tubers starch content is 65–85%. An important source of starch in many countries is sweet potato (*Ipomoea batatas*, Convolvulaceae), the Jerusalem artichoke, also called topinambours (*Heliantus tuberosus*, Asteraceae) and tubers of *Manihot esculenta* (Euphorbiaceae), an important source of food carbohydrates in the Tropics, known in Asia and Africa by the name cassava and in South America as manioc, yucca or tapioca. Another source of starch is sago (sago palm) obtained from the spongy pith of stems of various tropical palms, especially true sago palm (*Metroxylon sagu*, Arecaceae). The sago cycad (often also called sago palm) is obtained from the pitch of *Cycas revoluta* (Cycadaceae).

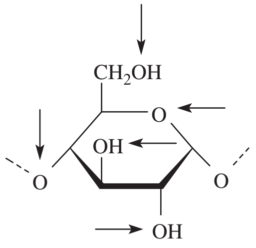
Ripe fruit, with some exceptions, does not contain starch. Bananas (*Musa* sp.) contain a small amount of starch, followed by sweet chestnuts, also known as marrons (*Castanea sativa*, Fagaceae) and various nuts. A higher starch content is found in the seed of the cashew tree (*Anacardium occidentale*, Anacardiaceae), commonly called cashew nuts.

Production of starch is relatively simple. Starch granules occurring in amyloplasts are not chemically or physically bound to other components of raw materials. Their density is high (around 1600 kg/m^3); therefore pure starch can be separated from the crushed material by either washing or decantation on sieves or by centrifugation.

4.5.6.1.3 Properties and changes

Gelatinisation

At ambient relative humidity, starch granules take about 0.2 g of water per 1 g of dry starch from the atmosphere and contain about 17% water (wheat starch is about 13% water and potato starch 18–22% water), without changing the volume of the grains. The process is called imbibition (see Section 7.8.3.1). One molecule of glucose binds 1.5 water molecules. The structural units of glucans contain a total of five oxygen atoms that can interact with water (**4-139**).



4-139, interaction of glucose unit with water molecules

Starch granules are insoluble in cold water and form a suspension. In cold water, the suspension of undamaged starch granules may absorb a small amount of water. Relatively large amounts of water are absorbed during heating without disturbing the integrity of the granules. Up to a certain temperature, called the initial **gelatinisation temperature** (T_o) at which swelling of granules occurs, it is a reversible process. The final gelatinisation temperature, when the gelatinisation process ends (T_e), the gelatinisation temperature range $T = T_e - T_o$ and the energy necessary to complete the process (gelatinisation enthalpy H_p) are also important starch characteristics. The initial gelatinisation temperature is generally 50–70 °C (Table 4.26) and depends on the starch origin, the ratio between starch and water, pH and the presence of other components (salts, sugars, lipids and proteins). The gelatinisation temperature range is usually 10–15 °C. It refers to the temperature range over which all the granules are fully swollen.

Table 4.26 Gelatinisation temperatures of selected starches.

Starch source	Gelatinisation temperature (°C)			Starch source	Gelatinisation temperature (°C)		
	Initial	Medium	Final		Initial	Medium	Final
Wheat	52	58	64	Rice	66	72	78
Maize	62	67	72	Potatoes	50	60	68
Waxy maize	63	68	72	Cassava	61	66	71

The changes to swollen starch granules in the process of gelatinisation are irreversible. Thermal motion of molecules interrupts the existing hydrogen bonds; water molecules penetrate the amorphous regions of granules and interact with free binding sites of the polymers. Hydrated polymer chains move away from each other, revealing new binding sites, which also interact with water; double helices of the side chains of amylopectin break down, which leads to the disappearance of crystalline zones, and the whole structure becomes disorganised and amorphous. Granules swell intensely and their volume increases. For example, the size of wheat starch granules at this stage can be up to 30 μm (Table 4.23).

Upon further heating, some amylose and amylopectin molecules (originally located in the granule in the radial direction) reach the surface. Linear amylose molecules (less bulky than amylopectin molecules) penetrate this tangentially located sieve of molecules and are released into the extragranular environment (partly also broken down into shorter molecules), where they are fully hydrated. A small proportion of amylopectin molecules are also released to the extragranular environment.

As a consequence of hydration at elevated temperatures (e.g. at 70 °C), granules absorb water of about 25 times their weight. For example, 1 g of dry potato starch can take up a volume of about 200 ml after swelling. The release of amylose from the granules increases the viscosity and at sufficient concentration (roughly in a 1% suspension), starch yields viscous **starch paste**. It contains collapsed starch grains, but magnified many times. They contain mainly molecules of amylopectin and any remaining molecules of amylose (e.g. wheat starch contains about 8% of the original amount of amylose after heating to 90 °C). The branched amylopectin molecules give viscosity to the cooked paste. Their side chains and bulky shape keep them close enough to bond together.

Additional heating results in decreased viscosity and further loss of integrity of granules. Viscosity gradually increases again when the solution is chilled because of renewing hydrogen bonds between the macromolecules of amylose and amylopectin, and continuous chilling makes the solution cloudy. Left to stand, the solution becomes white and in cases where there is a higher concentration of starch, it gels. The **starch gel** is a solid three-dimensional network that captures a large amount of water. Less concentrated starch suspensions form a viscous paste or viscous colloidal solution (Table 4.27). Starch gel is a complex system of gelatinised granules located in the matrix formed by amylose that does not contribute significantly to viscosity, but they contribute to the gel formation as the linear chains can orient parallel to each other, moving close enough together to bond. Amylose forms a gel at room temperature at concentrations higher than 0.9–1.0%. Amylose gels are thermally stable even at a temperature of 120 °C. Amylopectin molecules do not usually contribute to gel formation. Amylopectin forms gels only at concentrations higher than 10% and temperatures lower than 5 °C. The gel formation is slow and the formed gels are thermoreversible (they melt when heated to 40–60 °C), like starch gels.

The rheological properties of starch gels depend on the origin of the starch, the degree of granule degradation, the ratio between the interacting polysaccharides (amylose and amylopectin), temperature, the amount of water present and the type and amounts of other compounds. Cereal starches, for example, generally form turbid, opalescent gels. Gels formed from amylose starches are formed faster and at higher temperatures than gels from waxy cereal varieties. They are stronger and their strength increases with starch concentration, but they quickly undergo retrogradation and loss of water binding capacity caused by recrystallisation of amylose through cooling. Starches from waxy varieties of cereals, where amylopectin predominates, form gels with difficulty and only after cooling to low temperatures. These gels are clear, soft and often have a paste-like consistency. The linear segments of amylopectin also have a tendency to associate over time. Prolonged storage at low temperatures causes retrogradation, as in amylose gels. Clear and moderately firm gels are formed from potato starch. The viscosities of diluted dispersions of major polysaccharides and starch are compared in Table 4.27.

Retrogradation

Gelatinised starch is not in thermodynamic equilibrium, therefore the structure and rheological properties of starch gels, pastes and diluted dispersions change after several hours of storage. Intermolecular associations by hydrogen bonds between aligned chains of amylase lead to a loss of sites that bind water molecules, hence water binding capacity decreases. Gels and concentrated pastes get a rubbery texture and higher strength, dilute dispersions lose viscosity and precipitate, bound water is released and eliminated, which gives rise to a solid–liquid two-phase system. These changes are related to the properties of amylose (its recrystallisation) and only very little to the properties of amylopectin. This process is generally called **syneresis**. Syneresis in starch gels is known as **retrogradation**. The process of retrogadation is actually the opposite of gel formation. The rate and extent of retrogradation depend on

Table 4.27 Typical viscosity of starch and other hydrocolloid dispersions at 25 °C.

Polysaccharide	Dispersion concentration (% w/w)	Dynamic viscosity (mPa/s)	Polysaccharide	Dispersion concentration (% w/w)	Dynamic viscosity (mPa/s)
Wheat starch	10	10	Tragacanth	1	54
Pectin	1	50	Agar	1	4
Guar gum	1	3025	Carrageenan	1	57
Locust gum	1	59	Alginate (Na)	1	214
Gum arabic	10	17	Xanthan	1	2000

many factors, such as the origin of the starch (the amount and degree of polymerisation of amylose), temperature, water content and the presence of other components. For example, maize starch undergoes retrogradation more easily than potato starch.

During low temperature storage (below about −5 °C), retrogradation of starch gels containing 45–50% water is strongly inhibited. At temperatures ranging from −5 °C to just below room temperature, the retrogradation rate is higher than its rate at room temperature. Higher temperatures (32–40 °C) effectively suppress retrogradation and at temperatures around 65 °C and above, retrogradation does not occur at all. Therefore, waxy starches high in amylopectin, where the range of retrogradation is suppressed, are recommended for frozen foods. When stored at low temperatures, even gels of these starches lose clarity and water binding capacity, which is attributed to intermolecular associations of amylopectin side chains. Retrogradation is also influenced by water content in starch gel.[6] It occurs in gels containing 20–90% water. The greatest tendency to retrogradation is seen in gels with 45–50% water. In the presence of salts (sodium chloride) or sugars, the degree of retrogradation is lower. The effect of individual sugars varies and depends on many factors, such as concentration. Retrogradation is also suppressed in the presence of lipids that form inclusion compounds with amylose.

Changes in foods

Bread and other cereal products Along with starch, foods very often contain water, sugars (mono- and oligosaccharides), lipids, proteins, salts and acids that influence the formation of gels. Grain grinding mechanically damages about 5–10% of starch granules and during dough rising, amylases (also known as flour diastase) preferentially attack the damaged starch granules. Starch is partially hydrolysed by α-amylase (1,4-α-D-glucan glucanohydrolase) a β-amylase (1,4-α-D-glucan maltohydrolase) to maltose, which is hydrolysed to glucose by maltase (α-glucosidase, α-D-glucoside glucohydrolase). Endoglycosidase α-amylase randomly attacks α-(1→4) glycosidic bonds in amylose polymer yielding glucose, maltose and units of higher molecular weight, known as **linear dextrins** or **α-dextrins**, and is therefore known as dextrinogenic enzyme. Exoglycosidase β-amylase hydrolyses α-(1→4) glycosidic bonds of amylose molecules from the non-reducing end, splits off maltose molecules (if the chain has an odd number of glucose units, glucose and maltotriose are also formed) and is therefore known as saccharogenic enzyme. Amylopectin is attacked by both enzymes, but it is hydrolysed from about 50–60%, because amylases only hydrolyse the molecule to the point of branching and do not hydrolyse α-(1→6) glycosidic bonds. β-Amylase hydrolyses amylopectin from the reducing end to the branching points so that residues containing 2–3 glucose units remain unhydrolysed. Products of this concerted action of amylases are maltose and dextrins (maltodextrins). Highly branched dextrins of this type are called **limit dextrins** or **β-dextrins**. Limit dextrins are further hydrolysed gradually by pullulanase (1,6-amyloglycosidase or amylopectin 6-glucano hydrolase), which hydrolyses α-(1→6) bonds, and remnants of dextrins are further broken down by amylases.

Rheological properties of dough are mainly determined by the properties of gluten. The desired structure of the dough also arises partly as a result of interactions of swollen starch granules with gluten proteins, with pentosans, denatured proteins and starch gelatinisation. If dough contains a limited amount of water, starch is only partially gelatinised (raw starch with no water added does not undergo gelatinisation, but instead undergoes dextrinisation if heated). This condition exists in the production of bread and pastries (similarly in the cooking of potato tubers and opposed to cooking a pudding). Because the water content of the bread crumb stays virtually unchanged, the resulting degree of starch gelatinisation and crumb consistency depends on the amount of water used, but also on the origin of the flour (its chemical composition), the amount of fat, emulsifiers and other factors.

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[6]Gelatinised starch gel is completely amorphous with uniformly distributed water molecules. Retrogradation is a non-equilibrium recrystallisation process dependent on the temperature difference between storage temperature and the glass transition temperature (T_g). Above this temperature (when the water content is about 50%, T_g is about −5 °C) polysaccharide chains are mobile and their mobility determines the speed of association by hydrogen bonds that causes retrogradation. Water as a plasticiser affects the value of T_g of amorphous gels. At low water content, the T_g value is higher than room temperature and the amorphous gel occurs in a highly viscous glassy state. Recrystallisation increases with increased water content and is a maximum at water contents of 45–50%, as the mobility of molecules grows. At higher water contents, recrystallisation is lower due to the dilution of macromolecules.

During bread and thicker pastry baking, the enzymatic hydrolysis of starch by amylases proceeds for quite a long time (α-amylase is very thermostable and the optimum temperature is around 90 °C). Bread and pastry therefore always contain less starch and more products of its fission (dextrins and lower sugars) than dough. Sugars bind a certain proportion of water, which can further contribute to the formation of gel. The decrease of water activity results in increased gelatinisation temperature and reduced rate of gel formation, and also reduces its viscosity and strength. Slow baking at lower temperatures produces higher amounts of lower sugars. For example, pumpernickel, a very heavy rye bread produced in Germany (Westphalia), is baked very slowly (24–100 h at 100–150 °C). It is sweet as it contains up to 20% reducing sugars. They undergo non-enzymatic browning reactions and products of these reactions confer a bread crumb with a typical dark brown colour and distinctive flavour.

Starch in bread crust exposed to temperatures of 160–180 °C during baking is non-enzymatically hydrolysed into smaller units, which condense in larger molecules called **pyrodextrins** that contribute to bread flavour, colour and crispness. Pyrodextrins contain glucose units bound by α-(1→6) glycosidic bonds or (6→6) ether bonds, which are not cleaved by saccharases present in the digestive tract.

Lipids (fats and oils) and monoacylglycerols used as emulsifiers form inclusion compounds with amylose, slow down the swelling of starch granules and the extent of starch gelatinisation. For example, around 96% of starch is fully gelatinised in white bread, which is low in fat. Bakery products rich in fat, especially in the surface layers with lower water activity, contain a considerable proportion of ungelatinised starch. Small concentrations of sodium chloride have only limited impact on gel formation.

A typical symptom of retrogradation is aging (hardening) of bread and pastries. Re-heating of such bread or bakery products, for exampe in microwave ovens or during bread toasting, causes new starch gelatinisation, but such products harden very quickly.

Other foods Starch gelatinisation also occurs during hydrothermal processes, such as extrusion cooking. In some cases, the release of amylose from the granules into the environment is an undesirable phenomenon (e.g. during pasta and rice cooking), causing stickiness of the material. To prevent this in pasta products, monoacylglycerols or other emulsifiers are added. In rice, cooking is helped by the decantation of starch and addition of oil to cooking water.

Preservation of unripe fruits and vegetables with higher starch content (e.g. apples and ripe green peas) often gives turbid and viscous brines. Acidic solutions can cause hydrolysis of starch to dextrins, which is manifested by less viscous solutions. Hydrolysis of starch also occurs in puddings containing acidic fruit juices. The liquefaction of dressings and mayonnaises containing starch is likewise caused by the action of enzymes (saccharases) of fresh vegetables and spices.

4.5.6.1.4 Physiology and nutrition

Starch is a utilisable polysaccharide easily digested in the small intestine. In the digestion of starch, the enzymes α-amylase (in saliva), pancreatic α-amylase and isoamylase (isomaltase or glycogen 6-glucanohydrolase) cleave the α-(1→6) of amylopectin and glycogen.

Most starches belong to the category of quickly or slowly digestible starches and, under normal conditions, are completely hydrolysed in the small intestine. However, some starches are partially resistant to enzymatic hydrolysis or are unacceptable to the host amylolytic enzymes and rank among the non-utilisable polysaccharides that become the components of dietary fibre (e.g. cereal grains consumed raw and protected by cell walls, preventing access to amylases). Four types of resistant starch are recognised (Table 4.28). Resistance to digestion in the small intestine can be also lowered by the method of preparation and storage of food. For example, retrogradation of starch increases the resistance to amylolytic enzymes by about 1%. Resistant starches represent about 1% of the total amount of starches consumed. They pass through the colon, where they are partially metabolised (also in the appendix) and used by the colon microflora. Resistant starch can therefore be considered as a low energy material that has a theoretical energy value of only about 8–12 kJ/g (2–3 kcal/g), while the energy value of digestible starch is 17 kJ/g (4 kcal/g).

4.5.6.1.5 Use

Starches are important natural components of many food commodities, which significantly affect or determine their texture and

Table 4.28 Classification and examples of digestible and resistant starches.

Starch type	Characteristics	Small intestine digestion	Source
Quickly digestible	Digestible	Total	Freshly cooked starchy foods
Slowly digestible	Digestible	Slow, but total	Majority of row cereals
Resistent starch I	Physically inaccessible	Undigestible	Unprocessed grains, seeds and legumes
Resistent starch II	Natural granules	Slow, partial	Raw potatoes and bananas, high amylose maize
Resistent starch III	Retrograded	Partial or resistant	Boiled and stored potatoes, old bread, bread crust, legumes, cornflakes
Resistent starch IV	Chemically modified	Resistent	Not found in nature

functional properties. Native and modified starches are used as additives in many products. The commercial and technological uses of starch are extremely diverse. Native starches are used as fillers and thickeners, gelling agents, water binders, fat substitutes, carriers of odour-active compounds and stabilisers of foams or emulsions. Those applied directly include native starch granules, dispersed granules, extruded starch and films obtained by drying starch dispersions. Starches are also an essential raw material for the manufacture of modified starches, sugars and some sugar derivatives. Roughly half of the starches produced are used as natural or modified starches in food and feed production, the rest are used in many other industries (pharmaceutical, paper, textile, construction industries, in cosmetics and so on).

Modified starches

The use of native starches is limited due to their physical and chemical properties. Starch grains are insoluble in cold water, and to obtain starch dispersion it is necessary to cook the starch. Starch viscosity is often high and confers to products a rubbery and cohesive texture. Amylose containing starches form rigid, turbid and retrogradable gels; waxy starches form clear, but soft gels, which become turbid during storage at low temperatures. Furthermore, native starches are easily hydrolysed in acidic solutions, for example, in acidic sauces during prolonged heating. Native starches are therefore modified in various ways so that some of these undesirable properties are limited or the obtained products have desirable properties. Modified starches are divided into:

- **transformed** (converted, degraded) starches
- **cross-linked** starches
- **stabilised** starches
- **otherwise modified** starches.

Transformed starches

Transformed starches are obtained from native starches by:

- **acid hydrolysis** (starches modified by acids)
- **oxidation** (bleached and oxidised starches)
- **heating** (dextrinised starches).

Acid hydrolysis is carried out by heating concentrated aqueous dispersions of starch (36–40%) with dilute mineral acids (usually with <7% HCl or 2% H_2SO_4) for several hours at a temperature of 40–60 °C, which is lower than the initial gelatinisation temperature. The suspension of starch is then neutralised, washed with water and the starch separated by filtration and dried. Under these conditions, amylose and amylopectin are partially hydrolysed in the amorphous regions of granules and the product is a so-called **soluble starch** as the damaged granules swell in cold water and collapse when heated above the gelatinisation temperature (higher than that of the original starch). The obtained dispersion is less viscous (its fluidity increases), so that modified starches can be dispersed at higher concentrations. This is significant when starches are used as fillers, as saccharose or fat substitutes. Hot sols are clear and on cooling form cloudy, opalescent gels (relatively clear gels are formed from waxy starches) that are more stable than the unmodified starch gels. Starches modified by acids are used primarily in the manufacture of confectionery (gum drops and candy). Soluble starch in blends with native starch is used to prepare pudding powders.

Oxidation is used to produce two types of starch:

- **bleached** starch
- **oxidised** starch.

Bleaching is carried out in an aqueous suspension by mild oxidation with small amounts of peracetic acid, hydrogen peroxide, sodium hypochlorite (NaOCl), potassium permanganate ($KMnO_4$), ammonium peroxosulfate $(NH_4)_2S_2O_8$, chlorine or other oxidising agents. Under these conditions, starch oxidation is minimal, but accompanying colouring substances (especially carotenoids) are removed.

Oxidation is carried out in aqueous suspensions using the same oxidising agents in a weakly alkaline medium. For example, sodium hypochlorite oxidises the primary hydroxyl group at C-6 to a carbonyl group and further to carboxylic group (under the formation of glucuronic acid), the secondary alcohol groups at C-2 and C-3 are oxidised to formyl groups, through the ring opening between the C-2 and C-3 carbon atoms, and subsequent oxidation yields a dicarboxylic acid. Ring opening and oxidation of a formyl group in the reducing end of the molecule yields gluconic acid. To some extent, the secondary hydroxyl groups are oxidised to keto groups (Figure 4.9).

Oxidised starches form clear liquid sols with greater fluidity than native starches. Stable gels are formed by cooling with a reduced tendency to retrogradation. Carboxylic groups with electric charges of the same sign repel one another and the tendency of molecules to associate is lower. Oxidised starches have the same use as starches modified by acids. In addition, they are suitable for coating meat and fish, because the package adhesion is significantly higher in comparison with unmodified starches. Starches oxidised with nitric acid have found use in the textile industry.

Heating of native dry starch or starch acidified by diluted mineral acids (about 0.2% HCl, H_3PO_4 or H_2SO_4), depending on the dextrination conditions (heating at 100–200 °C for several minutes to hours) and type of starch yields three basic types of products:

- **white dextrins** (in more acidic media by short heating at lower temperatures)
- **yellow dextrins**
- **British gums** (in less acidic media).

The main reactions are hydrolysis (the products are mainly linear molecules branched at 2–3% of the glucose units) and

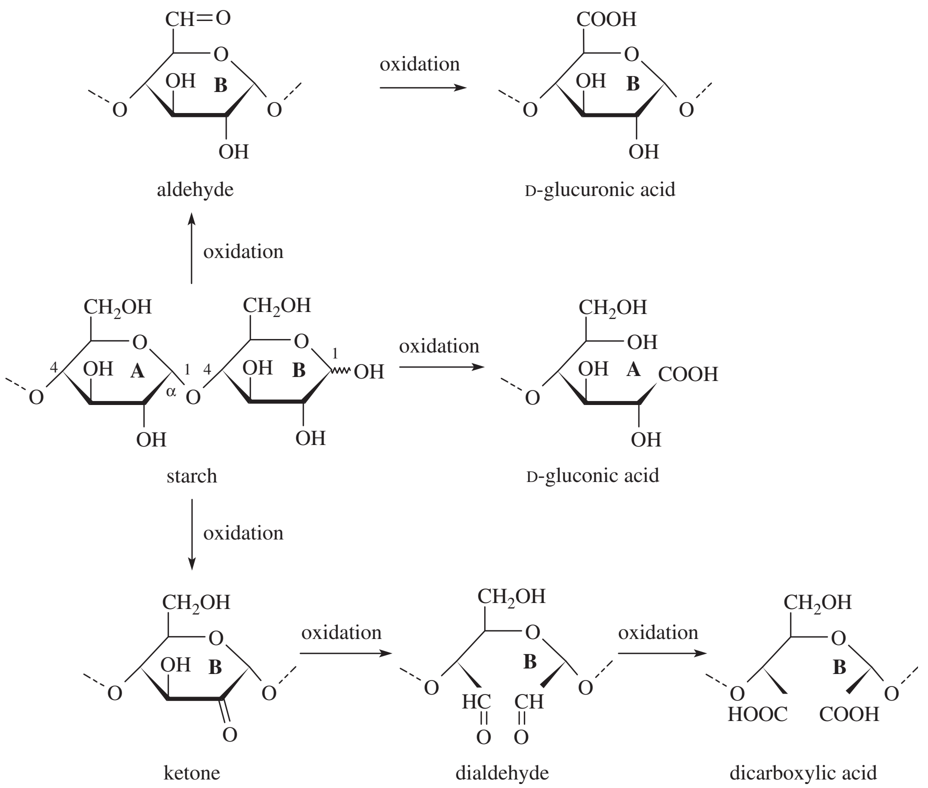


Figure 4.9 Major products of starch oxidation with sodium hypochlorite.

transglucosidation (yielding branched dextrins resistant to amylase attack). In later stages of the reaction, high molecular weight dextrins form by re-polymerisation of the formed products. The dispersions of white dextrins (hydrolysis predominates) are viscous and have the highest tendency to retrogradation. Yellow dextrins (hydrolysis prevails at the beginning of the process, later transglucosylation and finally polymerisation take place) contain about 20% branched molecules. British gums (the main reaction is transglucosylation) produce the most stable and least viscous dispersions containing 20–25% of branched molecules. Yellow dextrins and British gums are soluble in cold water (starch dispersions have to be prepared in hot water); the least soluble products are white dextrins. Dextrins find use as adhesive substances for the preparation of shiny surfaces of sweets and tablets, carriers, such as aromatic compounds, spices, dyes and for encapsulation of oils and water soluble flavours.

Cross-linked starches

Two main types of cross-linked starches are produced:

- **adipates**
- **phosphates**.

Adipates are prepared by reacting starch with adipic acid anhydride (mixed with acetic anhydride) in a weakly alkaline medium (Figure 4.10). Besides cross-links, the modified starch

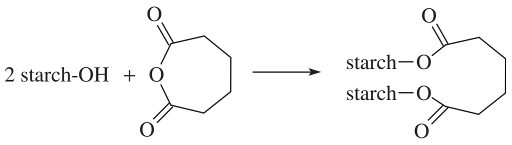


Figure 4.10 Formation of starch adipates.

contains small amounts of acetylated hydroxyl groups (especially at C-6). Reaction with phosphorus oxychloride ($POCl_3$) or sodium trimetaphosphate ($Na_3P_3O_9$) in alkaline medium is used for the preparation of distarch phosphates (Figure 4.11).

The degree of starch cross-linking is relatively low (usually one cross is in 1000–2000 glucose units), but it significantly modifies the rheological properties of starch. Swollen granules retain their integrity and the considerable viscosity of dispersions remains virtually unchanged. In contrast to cohesive pastes formed from unmodified starches, pastes of cross-linked starches are non-cohesive. Cross-linked starches are used for thickening, stabilising

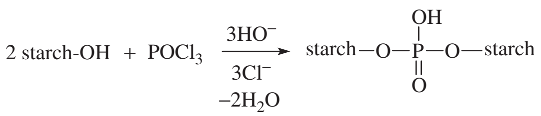


Figure 4.11 Formation of starch phosphates.

and adjusting the texture of foods (pastry fillings, dressings, thickening of soups and sauces), but they are not suitable for products stored at low temperatures. Other cross-linked starches (e.g. xanthates formed by reaction with carbon disulfide) are used in the textile industry.

Stabilised starches

The modification consists in the substitution of some hydroxyl groups of polysaccharides. Stabilised starches are divided into:

- **starch esters** (e.g. acetates, phosphates and succinates)
- **starch ethers** (such as hydroxyalkyl ethers).

Stabilised starches are usually made from native starches, but also from starches modified by other means (acid hydrolysis, dextrination and cross-linking). Acetylated starches are obtained by reaction of starch suspensions with acetic anhydride (also with vinyl acetate) in a weakly alkaline medium (Figure 4.12). They contain up to 2.5% of acetyl groups. The degree of substitution expressing the average number of substituted hydroxyl groups on each glucose unit is 0.1. The maximum number is 3 (Figure 4.9). The esterification occurs mainly at C-6 hydroxyl groups, less at C-3 and partially on C-2 hydroxyl groups. The modification is manifested by a reduced gelatinisation temperature of modified starches (especially of high amylase starches), higher stability in acidic media and higher stability against retrogradation during storage of products at low temperatures. The increasing degree of acetylation reduces the ability to form gels by cooling. Dispersions remain liquid even at higher concentrations of starch and at lower temperatures and in neutral and alkaline solutions are unstable (acetyl groups are hydrolysed). Acetylated starches (especially pre-cross-linked starches) are used for similar purposes as cross-linked starches.

Phosphorylated starches are obtained by reaction of aqueous suspension of starch with *ortho*- (Na_3PO_4), pyro- ($Na_4P_2O_7$) and tripolyphosphates ($Na_5P_3O_{10}$) in a weakly alkaline medium at temperatures of 120–170 °C (Figure 4.13). The degree of substitution is usually <0.25. The decrease in gelatinisation temperature versus

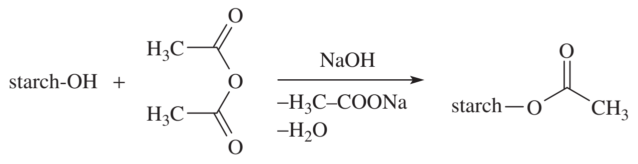


Figure 4.12 Formation of acetylated starches.

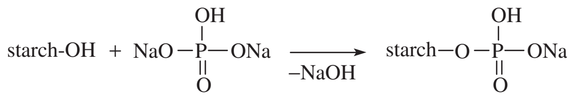


Figure 4.13 Formation of phosphorylated starches.

non-esterified starches is so great that even phosphates with a degree of substitution of 0.07 swell in cold water. Phosphorylated starches provide non-gelatinised dispersions with higher clarity, viscosity and stability at low temperatures. Like other polyelectrolytes, they interact with polyvalent cations (e.g. in hard water) under flocculation. Phosphorylated starches are used for thickening and stabilising unsalted, non-acidic (sodium salt) and stored refrigerated products, and for production of pudding powders soluble in cold milk.

Succinylated starches are prepared by reacting an aqueous suspension of starch with succinic anhydride or alk(en)ylsuccinic anhydrides in a neutral or slightly alkaline medium (Figure 4.14). The degree of substitution is 0.02 (one substituent to 50 units of glucose). Succinylated starches are used as thickeners. Some alk(en)ylsuccinylated starches have interesting properties, such as oct-1-enylsuccinated starch (Figure 4.14, $R = [CH_2]_6–CH{=}CH_2$), which is used as the sodium salt or acid. Some types are soluble in cold water and form a stable dispersion of higher viscosity than the original starch, which do not have a tendency to form pastes and gels. By the combination of hydrophobic substituent with ionised or non-ionised carboxyl groups, the starch has the functional properties of an emulsifier. It is used primarily to stabilise emulsions of oil-in-water in the pharmaceutical (e.g. encapsulation of vitamin A) and food industries (stabilisation of non-polar flavours in soft drinks, mayonnaises and dressings) or as a substitute for gum arabic.

For these and a variety of special non-food purposes esters with higher fatty acids, succinic, adipic and citric acids and carbamates (reaction products with urea), have also been prepared. Examples of starch ethers are 2-hydroxyethyl and 2-hydroxypropyl starches prepared by reaction of starch with oxirane (ethylene oxide) and methyloxirane (propylene-1,2-oxide). The reaction occurs preferentially at the secondary hydroxyl groups at C-2, with less on the C-3 and C-6 hydroxyl groups. The most common products are those shown in Figure 4.15. The degree of substitution tends to be <0.2. According to the reaction conditions, polyoxaalkyl starches[7]

[7] Instead the degree of substitution, these derivatives with polymeric substituents are characterised by the molar substitution, which is the number of moles of reagent bound per mole of (anhydro)glucose units.

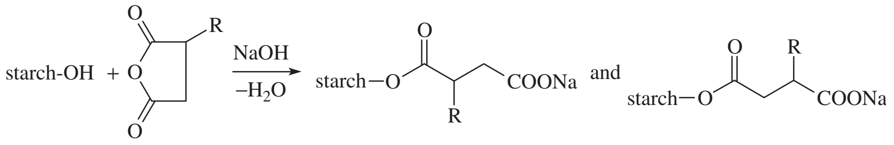


Figure 4.14 Formation of succinylated starches.

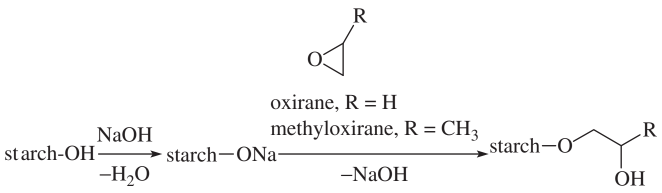
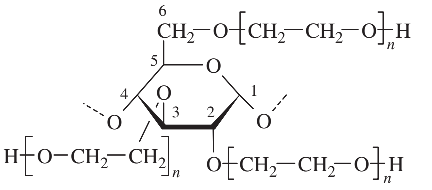


Figure 4.15 Reaction of starch with oxiranes.

substituted at positions O-2, O-3 and O-6 (**4-140**) can be also prepared.



4-140, polyoxaalkyl substituted starch derivatives

In the food industry, hydroxypropylated distarch phosphates are mainly used, providing a series of appropriate functional properties. They have greater stability at low temperatures, therefore they are used mainly for frozen products. They are suitable for products of low acidity (with pH 5–6), but also for acidic salad dressings. In acidic conditions and at higher temperatures they show a tendency towards partial hydrolysis, but the ether bond is generally more stable than the ester bond. Reaction with chloroacetic acid leads, by analogy, to carboxymethyl starches that swell quickly in cold water. They are used in the paper and textile industries (e.g. as glue). Reaction with chloroethane yields ethyl starches, reaction with ethenenitril (vinylcyanide) gives cyanoethylether starches, which also have various non-food uses. Quaternary ammonium and tertiary alkylamino groups are present in starches that are used as stabilisers of non-food dispersions.

Other modified starches

The functionality of modified (cross-linked and stabilised) starches can be increased by additional modifications, which usually include a combination of acid hydrolysis and dextrination, dextrination and cross-linking and other modifications. A possibility of increasing the functionality of modified starches is another modification by the action of enzymes. For these purposes pullulanase (isoamylase) is used, hydrolysing α-D-(1→6) bonds of amylopectin and dextrins. Such products are, for example, substitutes of caseinates in cheese imitations. Starches swelling in cold water or milk (used for the production of puddings) are prepared by drying of swollen starches called **pregelatinised starches**.

Physiology and nutrition

Modified starches are considered normal food components, because analogous products are formed during technological operations and starch digestion *in vivo*. Their digestibility is therefore comparable to the digestibility of native starches. Starches stabilised by esterification have comparable degrees of digestibility. Somewhat lower digestibility is shown by cross-linked starches and starch ethers.

Starch hydrolysates

In the past, starch hydrolysis was conducted exclusively by acids. At present, starch (native, less frequently modified, for example, pregelatinised) is hydrolysed by acids, enzymes (commonly several different enzymes are used) or combined methods (partially acid hydrolysed starch is hydrolysed enzymatically). Depending on the enzymes and technological procedures used, a number of products can be obtained. They are used as sweeteners, fat and sugar substitutes in low energy products, agents regulating texture and other properties of foods. Starch hydrolysates are also used as raw materials for production of other sugars and substances.

Acid hydrolysis

Partial hydrolysis with acid is carried out at higher temperatures than the production of soluble starch, generally in 40% starch suspensions containing HCl at a concentration of 0.02–0.03 mol/l at temperatures of 135–150 °C for 5–8 min.

Enzymatic hydrolysis and other reactions

Enzymatic hydrolysis provides similar products as acid hydrolysis (dextrins, maltooligosaccharides, maltose and glucose), but the resulting products are better defined and the process can be better controlled. Biotechnological procedures can be used to obtain products that cannot be provided by chemical hydrolysis (such as fructose and cyclodextrins). Amylolytic enzymes used for starch hydrolysis are exclusively of microbial origin. These enzymes hydrolyse α-(1→4) bonds in amylose and amylopectin with the formation of oligosaccharides of different chain lengths. The following basic groups of enzymes are:

- **endoamylases** (thermolabile and thermostable α-amylases)
- **exoamylases** (maltogenic β-amylase and glucogenic or amyloglucosidase or glucoamylase).

Thermostable α-amylases derived from bacteria of the genus *Bacillus* (*B. subtilis* var. *amyloliquefaciens* and *B. licheniformis*) break down starch into maltooligosaccharides. For example, the primary intermediate generated by α-amylase from *B. licheniformis* is linear maltopentaose, which is gradually degraded to lower carbohydrates. The main degradation product is maltotriose, formed by splitting off maltose, followed by maltose, glucose and maltotetraose. Maltotriose and maltose are the final products that are not further hydrolysed. These sugars are also the main products of thermolabile maltogenic α-amylase derived from the fungus *Aspergillus oryzae*. Maltogenic β-amylase breaks down starch (amylose) to maltose almost exclusively. The end product of hydrolysis of amylopectin by β-amylase only is called **maltodextrin**. This enzyme,

which also partly cleaves α-(1→6) bonds of amylopectin, can be obtained from bacteria of the genera *Bacillus* and *Pseudomonas* and from *Clostridium thermosulfurogenes*.

Glucogenic amyloglucosidase attacks α-(1→4) bonds of amylose and amylopectin molecules from the non-reducing end and splits off β-D-glucopyranose. It also partly cleaves α-(1→6) bonds of amylopectin. At high concentrations of glucose, this enzyme acts as transglucosidase and catalyses the reversion of glucose to maltooligosaccharides and isomaltooligosaccharides.

The α-(1→6) bonds of amylopectin and dextrins cleave α-(1→6)-saccharidases known by the trivial names of pullulanases or 1,6-amyloglycosidases or by the systematic name of amylopectin 6-glucanohydrolases. These bonds are also cleaved by isoamylases (isomaltases) known by the systematic name of glycogen 6-glucanohydrolases. Pullulanase and isoamylase are obtained from the bacteria *Klebsiella aerogenes*, and isoamylase also from bacteria of the genus *Pseudomonas*. Conversion of glucose into fructose is catalysed by glucose isomerase, which is obtained from *Bacillus circulans*.

Hydrolysis of starch (amylodextrins) to non-reducing cyclomaltooligosaccharides called cyclodextrins is catalysed by cyclodextrin glycosyltransferase (also known as CGTase). Cyclodextrin glycosyltransferase is produced largely by bacteria of the genus *Bacillus*. *B. macerans* synthesises mainly cyclomaltohexaoses (known as α-cyclodextrins), *B. stearothermophillus* produces cyclomaltohexaoses and cyclomaltoheptaoses (β-cyclodextrins) and the enzyme from *B. subtilis* yields cyclomaltooctaoses (γ-cyclodextrins).

Products of starch hydrolysis

The degree of hydrolysis of starch is characterised by the so called **dextrose** (glucose) **equivalents** (DE) that indicate the percentage content of free glucose, respectively, the content of terminal (reducing) glucose in maltose, maltotriose and other reducing maltooligosaccharides after conversion to dry matter. Native starch has the value of DE = 0, the hydrolysate containing only glucose has the value of DE = 100. Products with a DE value of about 20 or less form viscous solutions and have a sweet taste. The viscosity of starch hydrolysates decreases with the increased value of DE, while their sweetness (which ranges from 25 to 50% of saccharose sweetness) increases. A wide range of products with the values of DE within about 5–95 and more are produced. Depending on the extent of hydrolysis, these products contain variable amounts of glucose, maltose, maltotriose and higher glucooligosaccharides. The predominant components that can be identified are:

- **maltodextrins**
- **starch syrups** or **maltose syrups** or **glucose syrups**.

Maltodextrins are products of hydrolysis with a DE value of ≤20. They generally contain from 0.3 to 1.6% of glucose, 0.9 to 5.8% of maltose, 1.4 to 11.0% of maltotriose, 1.4 to 6.1% of maltotetraose and 75.5 to 96.0% of higher sugars. Hydrolysates containing maltodextrins are mostly dried or, exceptionally, prepared as syrups. They increase the viscosity, smoothness and surface gloss of confectionery products, prevent the formation of crystals in ice creams and frozen dairy products and are used as carriers for flavours, pigments and fats, as well as for replacement of gum arabic. Their main use is as fat substitutes.

According to their DE values, starch syrups are divided to type I (DE = 20–38), type II (DE = 38–58) and type III (DE = 58–73). Types II and III fall into the category usually called maltose syrups. Type IV (DE > 73) are glucose syrups. Their basic chemical composition is shown in Table 4.29. Starch syrups are used for making sweets, soft drinks, fruit syrups, jams, they serve as stabilisers of consistency (in ice creams), are used as substitutes for fat and as raw material for production of caramel and other products. They are fermentable and also serve in the biotechnological production of various substances (such as ethanol and citric acid). Maltose is isolated from syrups with a high maltose content. Isomerisation of maltose in maltose syrups in alkaline media is employed for the preparation of products that contain as the main component maltulose, which is sweeter than maltose. Hydrogenation yields sugar alcohol maltitol, correspondingly a mixture of alditols that contain as the essential components maltitol and maltotriitol. Glucose syrups are used for isolation of glucose and are also used for the production of fructose syrups. Hydrogenation yields a mixture of sugar alcohols, in which the main component is D-glucitol.

Fructose syrups are obtained by the action of bacterial enzyme glucose isomerase (from *Bacillus circulans*) on glucose syrups. Historically, the first products contain fructose in the amount of about 42% of dry matter (content of glucose was 52%, contents of higher sugars and dry matter was 6 and 75%, respectively). Today produced syrups contain 55% fructose (40% glucose, 5% higher sugars and 77% dry matter) and have a similar composition and similar sweetening power as invert sugar (about 100% relative to saccharose sweetening power). Using different physico-chemical methods, these syrups are used to produce high fructose syrups containing up to about 90% fructose (9% glucose, 1% higher sugars, 80% dry matter). Their sweetening power is 160–180% the saccharose sweetening power. Fructose syrups are used similarly to invert sugar and saccharose and are particularly suitable to sweeten soft drinks and confectionary. High fructose syrups are added to foods as a sweetener for diabetics.

Special products similar to maltose syrups are maltooligosaccharides containing 2–7 glucose units linked to each other by α-D-(1→4) bonds. They are obtained by enzymatic hydrolysis of starch with isoamylase or pullulanase and by hydrolysis of the obtained products using α-amylase. Maltooligosaccharides are fully utilisable sugars, which are used similarly to maltose syrups.

Other special products are isomaltooligosaccharides containing 2–5 glucose units linked by α-D-(1→6) and partly by α-D-(1→4) bonds, which occur in the trisaccharide panose. They are obtained by hydrolysis of starch by α-amylase and by the hydrolysis of the thus obtained products using β-amylase and α-glucosidase (α-D-glucoside glucohydrolase) under controlled conditions. They are not utilisable, but stimulate the growth of bifidogenic bacteria in the colon.

Glucose syrups are also transformed by enzymatic transglucosylation (using amyloglucosidase activity) to gentiooligosaccharides

Table 4.29 Composition of selected starch hydrolysates (% of dry matter).

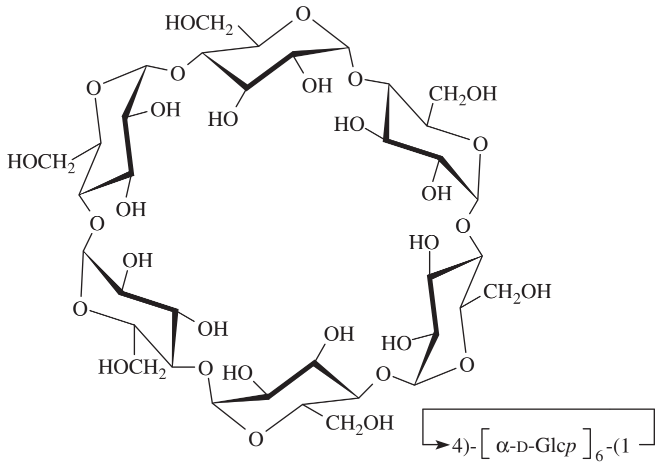
DE value[a]	Glucose	Maltose	Maltotriose	Maltotetraose	Higher saccharides
Acid hydrolysate 28	10	9	8	7	66
55	31	18	12	10	29
Enzymatic hydrolysate 5	0	1	1	1	97
10	1	4	6	5	84
Acid and enzymatic hydrolysate					
15/22	2	6	8	– [b]	84
10/95	93	– [c]	– [c]	– [c]	– [c]

[a] The first number for combined acid and enzymatic hydrolysis is the DE value after acid hydrolysis and the second number is the DE value after enzymatic hydrolysis.
[b] The content is included in the content of higher sugars.
[c] The total content of oligosaccharides is the sum of up to 100%.

that contain 2–5 glucose units linked by β-D-(1→6) glycosidic bonds that occur in the disaccharide gentiobiose (Table 4.11). They are used for the same purposes as other indigestible oligosaccharides, mostly as fillers in low-energy products. Like other indigestible oligosaccharides, gentiooligosaccharides also stimulate the growth of bifidogenic microflora in the colon.

Cyclodextrins

Cyclodextrins (formerly called Schardinger dextrins) are cyclic maltooligosaccharides to maltopolysaccharides containing 6–12 glucose molecules joined by α-D-(1→4) bonds. They are obtained by hydrolysis of amylodextrins (DE < 10) by the action of cyclodextrin glucanotransferase. The main representatives are cyclomaltohexaose (formerly Schardiner α-dextrin or α-dextrin, **4-141**), cyclomaltoheptaose (β-cyclodextrin) and cyclomaltooctaose (γ-cyclodextrin), which are composed of 6, 7 and 8 glucose monomers, respectively.



4-141, cyclomaltohexaose

Cyclodextrins form crystalline inclusion complexes with many organic compounds, including some gases, which are bound within the molecule. Formation of these complexes is called **encapsulation**. Encapsulation results in a change of the physico-chemical properties of encapsulated compounds (e.g. volatility of flavour-active compounds and their increased stability against oxidation and photodegradation). Cyclodextrins are, therefore, of greatest use as carriers (encapsulators) of odoriferous substances, emulsion stabilisers and are also used to remove bitter substances from citrus juices (see Section 8.3.5.1.1).

4.5.6.2 Other storage polysaccharides

Tubers, roots, seeds and also some vegetative parts of plants contain non-starch storage polysaccharides, which are involved in processes associated with germination and growth. Most of these polysaccharides are structurally similar to non-cellulose polysaccharides of cell walls that are classified as hemicelluloses and pectins. The most important representatives of this group of polysaccharides are:

- **heterofructans**
- **heteromannans**
- **heteroglucans**.

4.5.6.2.1 Heterofructans

Polymers and oligomers of D-fructose are called fructans or fructosans, but they often include D-fructose polymers containing one molecule of D-glucose as the terminal unit, the correct name for which is glucofructans or glucofructosans. Fructans and glucofructans are synthesised as energy reserves of about 15% in higher plants and some microorganisms (some species of the mould

genera *Aspergillus*, *Claviceps*, *Fusarium*, *Penicillium* and yeasts, such as *Saccharomyces cerevisiae* and others). In addition to being storage polysaccharides, they play a role in osmoregulation in plants and in their tolerance to some stress factors (cold and lack of water).

Structure

Natural fructans (glucofructans) are usually classified into:

- **inulins**
- **levans** otherwise called **phleins**.

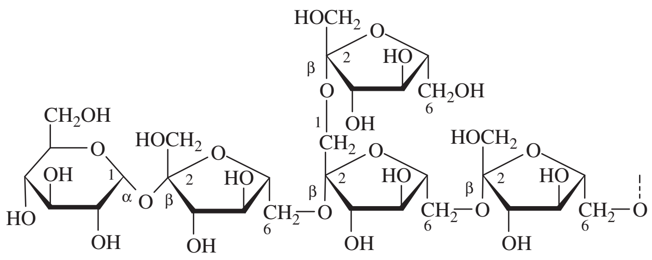
Polymers referred to as inulins are composed of linear chains of D-fructofuranoses (fructans) usually containing a D-glucopyranose molecule (glucofructans) as a terminal unit. They are mutually bound by β-(1→2) glycosidic bonds. Typically, inulin is found in some plants of the Asteraceae family, for example in chicory roots (*Cichorium intybus*), yacon roots (*Smallanthus sonchifolius*), native to the Andean region of South America, tubers of Jerusalem artichokes also known as topinambours (*Heliantus tuberosus*) and dahlias (*Dahlia* spp.) and the fleshy flower heads of globe artichokes (*Cynara scolymus*).

Levans are branched polymers of D-fructofuranoses associated exclusively by β-(2→6) glycosidic bonds. In addition to inulin, they may also contain D-glucose as the terminal unit. Typical levans are synthesised, for example, by bacteria *Bacillus subtilis* from saccharose or raffinose in sugar beet juice during sugar production. Levans with β-(2→6) glycosidic bond also predominate in oats.

Glucofructans that contain both types of bonds (1–4% of branched molecules) occur most frequently as components of numerous cereals (wheat, rye, triticale and barley), fruits and vegetables.

In the past, the name inulin was only used for polysaccharides of chicory, Jerusalem artichokes and dahlias. Inulin of asparagus (*Asparagus officinalis*, Asparagaceae) was called **asparogesin**. Trivial names were also used for some cereal fructans and glucofructans. Wheat levan with β-(2→6) bonds was called **pyrosin**, rye levan was **secalin**, wheat polysaccharide with both types of bonds was **triticin** and the corresponding polysaccharide of rye was known as **graminin**.

If the molecule end is α-D-glucopyranose, the abbreviated notation is GF_n (G = glucose, F = fructose, n = degree of polymerisation, **4-111**). The prototype of glucofructans of inulin type is actually disaccharide saccharose (GF, **4-109**). The enzymatic hydrolysis of inulin by native plant endoglycosidases, known as 2,1-β-D-fructan fructanohydrolase or inulase, provides linear polymers of type GF_n or, after hydrolysis of glucose, F_n type polymers. The latter type is a linear homopolymer of fructose, which always accompanies inulin. Low molecular weight sugars with a degree of polymerisation 2-10 are called fructooligosaccharides or oligofructoses (**4-112**). Typical microbial levans (of bacteria *Bacillus subtilis* and other bacteria) are of type G-F_n or F_m-G-F_n. The structure of a glucofructan of levan (phlein) type, or rather of a mixed type occurring in wheat and other grains, is shown in formula **4-142**.



β-D-Fru*f*
2
↓
1
α-D-Glc*p*-(1→2)-β-D-Fru*f*-(6→2)-β-D-Fru*f*-(6→2)-β-D-Fru*f*-(6→···

4-142, glucofructan of phlein type

Occurrence

Glucofructans together with oligofructoses (oligosaccharides formed by partial enzymatic hydrolysis) occur in many foods of plant origin. They are highly polydisperse mixtures of related compounds with a degree of polymerisation (number of bound fructose molecules) n = 2–60 and sometimes more. Therefore, they can be classified simultaneously as oligosaccharides and polysaccharides. In some plants oligosaccharides are exclusively present and higher polymers are absent. For example, levans of oats contain 2–5 monosaccharide molecules, the main glucofructan of onion is an oligomer with a degree of polymerisation n = 5, yacon roots are also of a low degree of polymerisation (n = 3–9) containing as the main component the GF_3 oligosaccharide, inulin of Jerusalem artichokes (topinambours) has an average degree of polymerisation n = 30, while the globe artichokes synthesise inulin containing up to 200 monosaccharide molecules. Glucofructans and fructans are commonly accompanied by saccharose (n = 1), fructose, glucose and other saccharides (Table 4.30). Some aminoacyl sugars, saccharoses acylated with amino acids (glycine, alanine, valine, threonine, tyrosine, tryptophan and histidine) have also been identified at the C-2 of the glucose moiety in sweet potato (*Ipomoea batatas*, Convolvulaceae).

Properties and changes

In the enzymatically active material, inulin is easily hydrolysed by endogenous inulase (inulinase) and the resulting monosaccharides are degraded during thermal processes. Examples could be changes in the content of inulin during storage and thermal processing of chicory root, which is used for the manufacture of coffee substitutes. When chicory root is stored at 5 °C, inulin is completely enzymatically hydrolysed to oligofructoses F_n (n = 3–7) within six weeks. Roasting of chicory root leads to extensive degradation of inulin, oligofructoses and higher reducing sugars, which caramelise and participate in the Maillard reaction with proteins and amino acids also present. The reaction is dependent on the temperature and roasting time. The optimum roasting time is about 55–60 min at 170 °C or 14–15 min at 175–200 °C. A good roasted product

Table 4.30 Content of glucofructans and fructans in the fresh edible part of some crops.

Source	Latin name	Glucofructans (%)	Fructans (%)
Asparagus	*Asparagus officinalis*	2–3	2–3
Bananas	*Musa* sp.	0.3–0.7	0.3–0.7
Chicory (root)	*Cichorium intybus*	15–20	8–11
Garlic	*Allium sativum*	9–16	3.5–6.5
Globe artichokes	*Cynara scolymus*	3–10	0.3–1
Jerusalem artichokes	*Heliantus tuberosus*	16–20	12–15
Onion	*Allium cepa*	1.1–7.5	1.1–7.5
Pore	*Allium ameloprasum*	3–10	2.5–8
Rye	*Secale cereale*	0.5–1	0.5–1
Wheat	*Triticum aestivum*	1–4	1–4
Yakon	*Smallanthus sonchifolius*	10–12	-

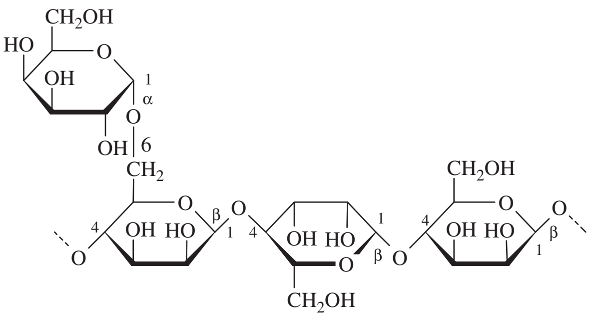
should then contain 13–20% of fructose and 28–32% of the original amount of inulin (a fraction with a lower degree of polymerisation than that of original inulin).

Physiology and nutrition

Glucofructans and fructans are physiologically acting polysaccharides or oligosaccharides that are classified as dietary fibre because they are not hydrolysed in the upper gastrointestinal system by saliva and pancreas hydrolases and by intestinal hydrolases (α-amylase, saccharase, maltase and other saccharidases). They also act as a growth factor for bifidobacteria in the colon. The daily intake of inulin and its hydrolytic products per capita in developed countries is estimated at 2–12 g.

Use

Inulin extracted from Jerusalem artichokes or chicory roots was used as the raw material to produce fructose syrups for diabetics and for the production of fructooligosaccharides. The main raw material for the production of fructose syrups is now starch and fructooligosaccharides are mainly produced from saccharose.



α-D-Gal*p*
1
↓
6
····→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→4)-β-D-Man*p*-(1→····

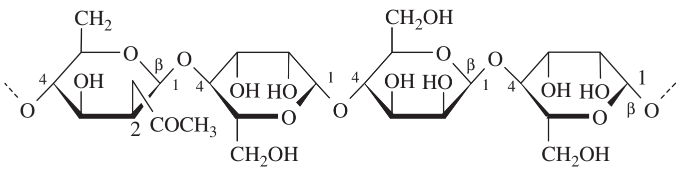
4-143, basic structure of galactomannans

4.5.6.2.2 Heteromannans

In addition to starch and heterofructans, the storage polysaccharides of some seeds are heteromannans. Their main chain is homopolymeric and formed by D-mannose units being linked to each other by β-(1→4) glycosidic bonds. Some of the mannose units are bound by (1→4) bonds to α-D-galactose. These heteromannans are therefore called **galactomannans** (**4-143**).

Galactomannans that differ in their degree of substitution of the main chain by D-galactose (4–96% of mannose units are substituted) are found in seeds of many plants. Palm seeds (e.g. seeds of dates) contain galactomannans, in which about 4% of mannose units are substituted. Related galactomannans with a degree of substitution of 30–96% are located in the endosperm of coffee seeds and in some legumes. The most important representatives of galactomannans, which have found use as food hydrocolloids, are **guar gum** and **locust gum**.

Related polysaccharides are linear copolymers, in which some of the mannose units are randomly substituted by residues of D-glucopyranose bound by β-(1→4) bonds. These heteromannans are called **glucomannans**. The ratio of mannose and glucose varies from 1.5 to 4. About 2–6% of sugars in the main chain are substituted by β-D-galactopyranose bound by (1→3) glycosidic bonds. Mannose residues (every fifth to twentieth) may be acetylated in the C-2 and C-3 positions (**4-144**). Glucomannans are found in varying amounts in plant tubers, roots and seeds. The only glucomannan that is used as a food hydrocolloid is **konjak gum**.



····→4)-β-D-Man*p*2Ac-(1→4)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Man*p*-(1→····

4-144, basic structure of glucomannans

Guar gum

Guar gum (guaran) is obtained as the flour from the endosperm of guar bean seeds (*Cyamopsis tetragonoloba*, Fabaceae) after separating the germ and the surface layer. The plant is native to Central Africa, but today it is grown primarily in India, Pakistan and the United States (Texas). Guar gum is a neutral galactomannan, whose primary structure is shown in formula **4-143**. Approximately every second residue of D-mannose is substituted by a D-galactose residue (ratio of mannose and galactose varies from 1.5 to 1.8). Minority sugars are D-glucose, D-xylose and L-rhamnose (Table 4.31). The average molecular weight of guar gum polysaccharides is usually in the hundreds of kilo daltons.

Guar gum is soluble in water giving highly viscous solutions stable in the pH range 4–10. Gel is formed in the presence of small amounts of borates. Guar gum can be combined with almost all natural gums, starches, pectins, cellulose and its derivatives and very often is combined with xanthan gum, which increases (as a synergist) viscosity of dispersions.

Guar gum has a very wide range of applications and is the most commonly used gum of plant seeds. It is used as a thickener, viscosity modifier and stabiliser of dispersions in foods and beverages. It is used not only in the food industry, but also in the paper industry as a glue, and is often used in cosmetics.

Locust gum

Locust gum, also called locust bean gum, carob, carobin or algarroba, is obtained as flour from the endosperm of seeds of the carob tree also known as St John's bread (*Ceratonia siliqua*, Caesapliniaceae). The tree comes from the Western Mediterranean region (Southern Europe, Northern Africa), but now grows mainly in Spain and in the subtropical regions of the United States and Australia.

Locust gum is galactomannan (**4-143**), as is guar gum, and substituted on approximately every fourth mannose unit by galactose residues (the ratio of mannose and galactose is 3.6 to 4.2; Table 4.31). The average molecular weight is approximately 310 kDa. The galactomannan content in the flour is around 88%.

The properties of locust gum are similar to those of guar gum, but the flour from locust beans in cold water only swells and is insoluble, although a viscous dispersion (of somewhat lower viscosity than a dispersion of guar gum) is obtained by heating. Locust gum is compatible with the majority of plant and microbial hydrocolloids and proteins (gelatine). It (as guar gum) does not form gels, but increases the elasticity and strength of agar and carrageenan gels, but a gel is formed with xanthan, which does not form a gel itself. In acidic media, locust gum is prone to hydrolysis.

Locust gum binding water is used as an emulsion stabiliser (e.g. in meat products), as a thickening agent for dairy products, and fillings for frozen foods and bakery products as it bounds water. Like guar gum, locust gum is not suitable for the preparation of clear dispersions as it contains insoluble residues of seeds. It is also used in the manufacture of cosmetic and pharmaceutical products. Locust gum is a relatively expensive hydrocolloid, so it is frequently substituted with cheaper guar gum.

Konjak gum

The only commercially important glucomannan is konjak gum also known as konjak mannan. Konjak flour is obtained from the starchy tubers of the plant *Amorphophallus konjac* (Araceae), grown in subtropical to tropical eastern Asia (Japan, China and Indonesia).

The basic structure of konjak gum polysaccharides is represented by formula **4-144**. The dominating sequences are composed of one, two and five molecules of mannose and one and two molecules of glucose. The ratio of mannose and glucose is around 1.6 and the acetyl groups content is about 15%. The average molecular weight is high, around 6000 kDa.

Konjak mannan dissolves in water to give highly viscous dispersions, which are pseudoplastic systems. Heating with a small

Table 4.31 Basic composition of guar and locust gum monosaccharides.

		Neutral sugars (% w/w)			
Gum	Latin name of plant	L-Arabinose	D-Glucose	D-Mannose	D-Galactose
Guar	*Cyamopsis tetragonoloba*	1.3–2.2	1.5–4.5	45.6–56.5	28.6–37.2
Locust	*Ceratonia siliqua*	0.8–1.4	1.5–2.7	59.3–69.9	16.0–18.2

amount of alkaline agents gives (under deacetylation) longer blocks of an unsubstituted polymannose skeleton capable of mutual associations with the formation of thermostable elastic gels.

Konjak flour is used mainly in East Asian countries as a thickener and gelling agent, especially in traditional Japanese cuisine for preparing noodles and gels, which are insoluble even in boiling water. It is also used for the preparation of edible films and coatings, which are semi-permeable to water vapour and oxygen. It acts synergistically with κ-carrageenan, xanthan gum and starch and affects the viscosity of their dispersions and structure of the gels.

4.5.6.2.3 Heteroglucans

Other plant storage polysaccharides are **xyloglucans**. Their primary structure consists of a chain of D-glucose units linked by β-(1→4) bonds. The chain branches consist of D-xylopyranose units linked by α-(1→6) bonds. The C-2 of about one half of the xylose units are connected to β-D-galactose molecules by glycosidic bonds (**4-145**). These xyloglucans are related to structural xyloglucans of cell walls (components of hemicelluloses), but they are not bound to cellulose and are extractable with hot water. The only xyloglucan that is used as a food hydrocolloid is **tamarind gum**.

Tamarind gum

Tamarind gum is a xyloglucan (**4-145**) located in the endosperm of the seeds of the tamarind tree (*Tamarindus indica*, Fabaceae), probably originating from Africa, but now grown in tropical regions on other continents.

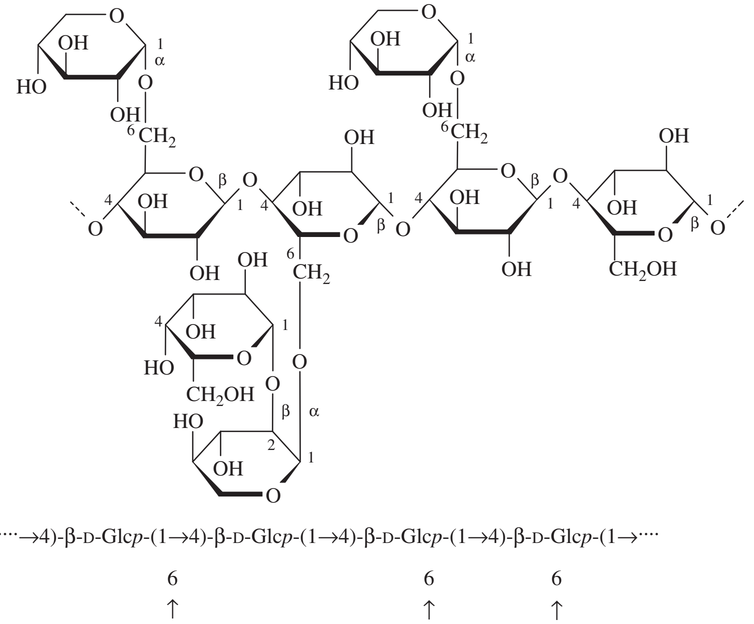
The viscosity of dispersions is increased by heating. Under acidic conditions, however, the tamarind gum polysaccharides undergo rapid hydrolysis. In the presence of high concentrations of saccharose (>65%) and over a wide range of pH values, tamarind gum forms gels that are stronger than pectin gels. Tamarind gum is only rarely used in food production, except in some special applications (as a thickener and foam stabiliser). The main use of tamarind gum is in the textile industry.

4.5.6.3 Cellulose

Cellulose is the most widespread natural organic compound. It occurs as a basic structural polysaccharide in the cell walls of higher plants. Cellulose is also found in green algae, fungi and, exceptionally, in cell walls of simple marine invertebrates (tunicates of the subphylum *Tunicata*).

4.5.6.3.1 Structure

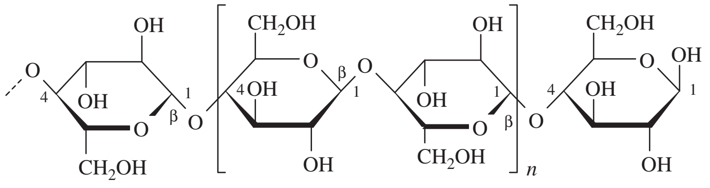
Homoglucan cellulose (**4-146**) is a high molecular weight linear polymer of D-glucose units linked by β-(1→4) glycosidic bonds. Each of the glucose units bound in the chain is turned towards the previous unit and this position is maintained by intramolecular hydrogen bonds between the hydroxyl group at C-3 and oxygen of the pyranose ring and the hydroxyl groups at C-2 and C-6 (**4-147**).



····→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→····

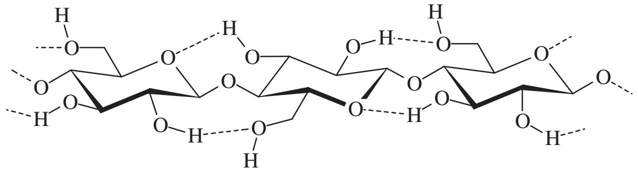
6	6	6
↑	↑	↑
1	1	1
α-D-Xyl*p*	α-D-Xyl*p*	α-D-Xyl*p*
	2	
	↑	
	1	
	β-D-Gal*p*	

4-145, basic structure of storage xyloglucans in seeds



β-D-Glc*p*-(1→4)-[β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)]$_n$-β-D-Glc*p*

4-146, cellulose primary structure



4-147, linear cellulose chain stabilised by hydrogen bonds

The degree of polymerisation is as high as 15 000. Individual cellulose macromolecules interact with each other through hydrogen bonds to form more or less organised three-dimensional structures in the walls of plant cells, called cellulose fibres or cellulose **microfibrils**, with high tensile strength. They have a thickness of approximately 10–20 nm, a length of several μm and contain about 30–100 cellulose macromolecules that are arranged in parallel in microfibrils and form a planar unit (sheet). Individual sheets are composed so that they are alternately shifted by half the length of the glucose units. The arrangement is stabilised by intermolecular hydrogen bonds between the glucose units in adjacent sheets (between oxygens of the pyranose rings and hydroxy groups at C-6). Such arranged areas of microfibrils with high numbers of intermolecular bonds are crystalline; less structured areas with a low degree of interactions are amorphous (Figure 4.16). The crystalline and amorphous regions in the microfibrils alternate. A higher proportion of crystalline areas are in the microfibrils of secondary cell walls (e.g. in wood); amorphous regions predominate in primary cell walls found in the flesh of fruits and vegetables. Compared with starch, cellulose is much more crystalline and several crystalline structures (allomorphs) are recognised. Cellulose I is natural cellulose, cellulose produced by bacteria and algae is mainly a cellulose I_α allomorph, while cellulose I_β is mainly found in higher plants.

Metabolically active (primary) cell walls of plant tissues have a common structure consisting of randomly oriented cellulose microfibrils with a predominantly amorphous structure. They make the major contribution to the mechanical strength of the plant

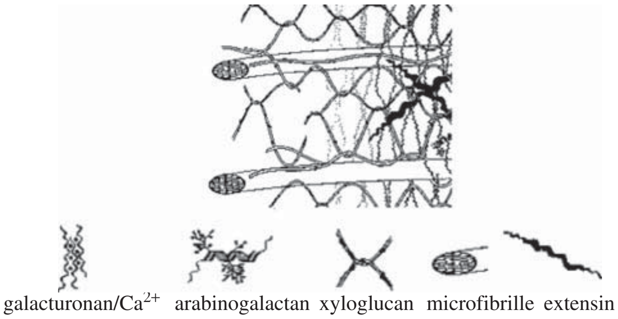


Figure 4.17 Schematic structure of plant cell walls. Carpita and Gibeaut, 1993. Reproduced by permission of John Wiley and Sons.

cell walls and act as the framework thereof. Microfibrils are bound through interactions and covalent bonds by a gel matrix composed of other structural polysaccharides, called non-cellulose polysaccharides (hemicelluloses and pectins), lignin and polypeptides, such as extensin (Figure 4.17) to form a biocomposite. The most important hemicelluloses in fruits and vegetables are xyloglucans, while cereals contain mainly arabinoxylans and β-glucans.

Differences in the structure of cell walls of various plants and their parts are related to the differentiation of the primary cells, cellulose crystalinity and the type and amount of non-cellulose polysaccharides. Deposition of additional layers of cellulose (in the form of clearly oriented parallel microfibrils), deposition of non-cellulose polysaccharides and lignification of the polysaccharide network, which is due to polymerisation of phenolic compounds, results in the formation of thick secondary cell walls, which have various special functions. For example, they ensure the rigidity of tissues, transport of water and have protective and other functions.

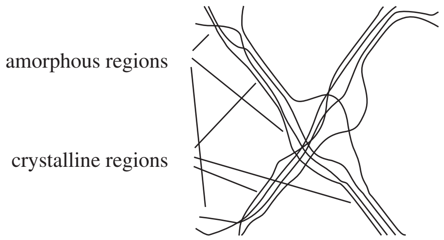


Figure 4.16 Amorphous and crystalline cellulose.

4.5.6.3.2 Occurrence

Cellulose represents a significant proportion of non-starch polysaccharides in foods (Table 4.20) and forms the so-called insoluble fibre (Table 4.21). Fruits and vegetables contain around 1–2% of cellulose, cereals and legumes 2–4%, wheat flour contains only 0.2–3% of cellulose, depending on the milling process (Table 4.19), because a large proportion of cellulose is found in the bran that contains 30–35% of cellulose. Cellulose also forms about 40–50% of wood mass, 80% of linen fibres and 90% of cotton fibres.

4.5.6.3.3 Properties

Cellulose is insoluble in water, dilute acids, bases and most solvents. Solvents, however, penetrate into the more accessible amorphous

regions of microfibrils, which leads to swelling, but the degree of swelling is always lower than that of starches. Cellulose can be dissolved in concentrated acids, because, depending on the conditions (acid concentration, temperature), it is hydrolysed to soluble fragments with shorter chains, such as disaccharide cellobiose, or to glucose. Swelling in solutions of hydroxides is greater than swelling in water or in acidic solutions. At elevated temperatures cellulose is hydrolysed or oxidised.

4.5.6.3.4 Physiology and nutrition

Cellulose is hydrolysed by a complex of cellulolytic enzymes of certain microorganisms (bacteria and moulds) and higher fungi called cellulases that break down plant tissues. Cellulase or *endo*-1,4-β-glucanase (1,4-β-D-glucan 4-glucanhydrolase) is an endoenzyme that splits β-(1→4)-D-glucosidic bonds in the amorphous regions in cellulose microfibrils with the formation of glucooligosaccharides and cellobiose. Cellobiohydrolase, also known as *exo*-β-D-1,4-glucanase (1,4-β-D-glucan cellobiohydrolase or avicelase) is an exogenous enzyme that splits β-(1→4) bonds from the non-reducing end of the chain to form oligosaccharides and cellobiose. Another enzyme, β-glucosidase or cellobiase (β-D-glucosid glucohydrolase), splits off cellobiose from the partially hydrolysed cellulose and higher oligosaccharides are hydrolysed to β-D-glucose.

Vertebrates have their own cellulase, but the digestive tracts of herbivores contain symbiotic bacteria that produce cellulolytic enzymes. Cellulose is therefore a utilisable polysaccharide for polygastric animals. It is broken down into glucose, which is fermented by bacteria to give lower fatty acids that are absorbed and utilised by the animals. Monogastric animals, including human beings, do not have cellulolytic enzymes and cellulose is a non-utilisable polysaccharide. Together with other polysaccharides, known as dietary fibre, cellulose is a nutritionally important and beneficial component of foods.

4.5.6.3.5 Use

Native cellulose is added to some foods as a non-calorific thickener, causing turbidity and is added to products processed by extrusion. However, **modified celluloses** have more applications in the food industry. Cellulose can be modified by either physical or chemical processes.

Modified celluloses

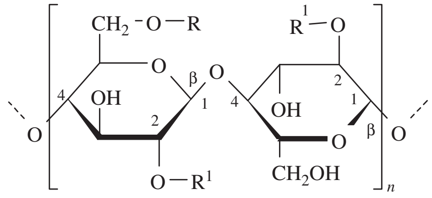
Physical modification (influence of high tangential stress and pressure) leads to cleavage of cellulose microfibrils and the product, with a high ability to bind water, is called **microfibrillar cellulose**. It can be used in food products as a non-calorific thickening agent and flavour carrier, in skin creams, paints and as a carrier for medicines.

The two main groups of chemically modified celluloses are:

- **hydrolysed cellulose**
- **derivatised cellulose**.

The only representative of hydrolysed cellulose is **microcrystalline cellulose**. It is obtained by partial hydrolysis of cellulose with hydrochloric acid, which dissolves the amorphous zones, but the crystalline zones remain unchanged. The product is most commonly known under the trade name Avicel, which has properties of thixotropic and pseudoplastic systems. Viscosity is independent of temperature and pH. Functional properties remain constant even at high temperatures and in acidic media (during baking, microwave heating and UHT processes). Microcrystalline cellulose is used as a dietary fibre, low-energy bulking agent, carrier of flavourings, stabiliser of foams and in extrusion technologies.

Of the many derivatives of cellulose, only some cellulose ethers have been used in food technologies. The most commonly used derivative is **carboxymethylcellulose** (its sodium salt), followed by **methylcellulose** and **hydroxypropylcellulose** (**4-148**).



4-148, cellulose ethers

R = CH_2COONa, R^1 = H, carboxymethylcellulose (sodium salt), degree of substitution 1.0
R = R^1 = CH_3, methylcellulose, degree of substitution 2.0
R = R^1 = $CH_2\,CH(OH)CH_3$, hydroxypropylcellulose, degree of substitution 2.0

The polyelectrolyte carboxymethyl cellulose (sodium salt) is produced by reaction of cellulose with chloroacetic acid in alkaline solution (NaOH). The degree of substitution ranges from 0.4 to 1.5. In solutions of $pH < 4$, carboxymethyl cellulose is present mainly as the free acid of low solubility that gives turbid solutions with divalent cations, such as Fe and Zn (salts are poorly soluble). In the presence of trivalent ions (Al and Fe), carboxymethyl cellulose forms gels and precipitates. Dispersions are non-Newtonian fluids that behave as pseudoplastic or thixotropic systems according to the degree of substitution. Carboxymethylcellulose is used as a thickener (cottage cheeses and cheese spreads), emulsion stabiliser (sauces, soups and dressings), protein solubiliser (gelatine and casein) and retarder of crystal formation (ice creams). Methylcellulose is obtained by cellulose methylation with methyl iodide in alkaline solutions. Reaction with propylene oxide yields hydroxypropylcellulose. The degree of methylcellulose substitution is <2.0, the degree of molar substitution of hydroxypropyl derivatives extends up to 4.0. A common cellulose ether is also hydroxypropylmethylcellulose (hypromellose), which contains two functional groups. It is used in the manufacture of pharmaceutical tablets. Less common is ethylmethylcellulose, which contains methoxyl and ethoxyl functional groups. The products are known, for example, under the trade name Methocel.

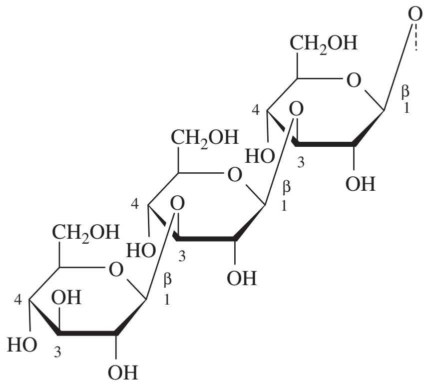
Unlike other gums, cellulose ethers show thermal gelatinisation as their colloidal solutions (at concentration of >1.5% by weight) form gels by heating to 50–85 °C. On cooling these gels

melt to viscous colloidal solutions, which are non-Newtonian pseudoplastic systems. Gels are formed in the presence of inorganic salts (phosphates and sulfates).

Cellulose ethers are used as thickeners, stabilisers of emulsions (in salad dressings) and foaming agents (hydroxypropyl cellulose). They can be added to bread to increase its water binding capacity and to reduce fat absorption by the product (e.g. of donuts during frying), to slow the retrogradation of frozen products and to produce edible films (gels) that protect, for example, frozen products against dehydration (desiccation).

4.5.6.4 Callose

Callose is an amorphous β-(1→3) glucan accompanying cellulose in plant cells walls, but it is less common than cellulose. Most plants synthesise this polysaccharide in response to injury or only at certain stages of development of cell walls. The basic building unit of callose is disaccharide laminaribiose, β-D-glucopyranosyl-(1→3)-D-glucopyranose (**4-149**).



4-149, callose

β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-β-D-Glcp-(1→....

4.5.6.5 Hemicelluloses

The term hemicellulose is a common name for non-cellulose structural polysaccharides of cell walls of plants that fill the spaces between the cellulose fibres (Figure 4.17). Hemicelluloses include two main groups of polysaccharides:

- **heteroglucans**
- **heteroxylans**.

Plant cell walls contain a large number of other polysaccharides that can be included under the term dietary fibre. Other important hemicelluloses are **heteromannans** (galactomannans and glucomannans) that are present in lower amounts in plant cell walls. They constitute about 12–15% of cell wall polysaccharides in the flowering plants (angiosperms). Their role as the building components of cell walls is still not well known. In larger quantities, heteromannans are present in some legume seeds, where they have the function of storage polysaccharides. Structures of heteromannans are illustrated by formulae **4-143** and **4-144**.

4.5.6.5.1 Heteroglucans

The main structural heteroglucans that are classified as hemicelluloses are:

- **xyloglucans**
- **β-glucans**.

Xyloglucans

The basic structural unit of the xyloglucan molecule is β-D-(1→4)-glucan (cellulose), with units of D-xylopyranose in side chains that are bound to glucose by α-(1→6) glycosidic bonds. Unlike the related reserve xyloglucans present in seeds of plants (**4-145**), structural xyloglucans contain the residue of D-galactose bound to D-xylose near the reducing end of the polysaccharide, which is bound by β-(1→2) bonds. They may also contain L-fucopyranose linked to an L-galactose by an α-(1→2) bond (**4-150**).

Xyloglucans of the hemicellulose type (structural xyloglucans) are the dominant hemicelluloses of cell walls of dicotyledons (dicots), plants that include most vegetables, root crops, legumes and fruit trees. The xyloglucans content is a characteristic of various tpyes of fruits. For example, their content in strawberries is 14.6–15.9 g/kg depending on the variety, cultivation and degree of maturity. Xyloglucans are therefore a good indicator of the amount of fruit present in various products, for example in fruit yoghurts.

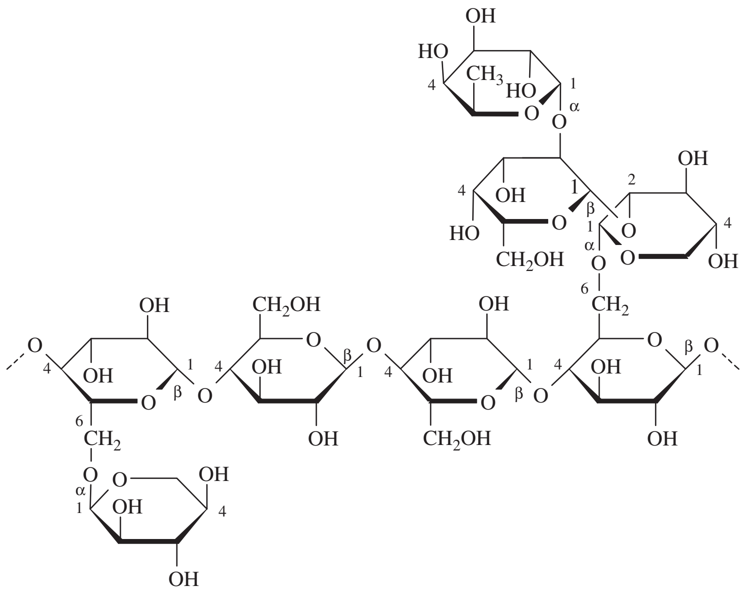
Xyloglucans are present in small amounts in monocotyledon plants (monocots) that include some vegetables (e.g. onion, garlic and asparagus) and, especially, cereals. The greater portion of the xyloglucans is a component of insoluble fibre.

β-Glucans

Polysaccharides known as β-glucans or β-(1→3), (1→4)-D-glucans or mixed linkage β-glucans (formerly lichenins) are found in negligible amounts in the cell walls of dicot plants, but in larger quantities are present in the cell walls of cereals, where they constitute up to 30% of dry matter of non-starch polysaccharides. While their content in wheat and rye is only 0.2–2% by weight of grain and 1–2% in unhulled rice, the European and American varieties of oats contain 3.2–6.8% of β-glucans and their content in barley is 3–7%. Some cultivars of barley contain even 14–16% of β-glucans.

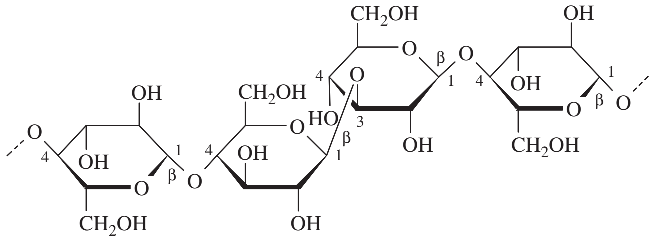
The oat bran contains β-(1→3), (1→4)-D-glucan, which is also called oat gum. Typical β-glucans for barley have two or more neighbouring (1→4) linkages (**4-151**). β-D-Glucans contain small quantities of arabinosyl and xylosyl residues. The relative molecular weights of β-glucans vary over a wide range, according to their origin, from tens to thousands of kilo daltons. Similar polymers, also called β-glucans or β-(1→3), (1→6)-D-glucans or mixed linkage β-glucans, are synthesised by higher fungi, moulds and yeasts.

β-Glucans can be partly soluble or partly insoluble dietary fibre. Solubility in water to give viscous solutions depends on their structure, which is related to the origin and decreases in the order:



α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*
1
↓
6
→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→····
6
↑
1
α-D-Xyl*p*

4-150, basic structure of xyloglucans of hemicellulose type



····→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→····

4-151, basic structure of mixed linkage β-glucans containing (1→3), (1→4) bonds

barley > oats > wheat. The more (1→4) bonds is in the molecule, the lower is the solubility of the polymers. More soluble polymers contain about 30% of (1→3) bonds and 70% of (1→4) bonds, and their chain is composed of 2–3 units of β-D-glucose linked by (1→4) bonds, between which is located a unit linked by a (1→3) bond. Solubility of β-glucans increases with temperature. For example, at 40 °C only about 20% of barley β-glucans can be extracted, while at 65 °C this amount increases to 30–70% (β-glucans of wheat are not extracted at this temperature). Native molecules do not form gels; however, a gel is formed after partial hydrolysis. β-Glucans bound to proteins are insoluble.

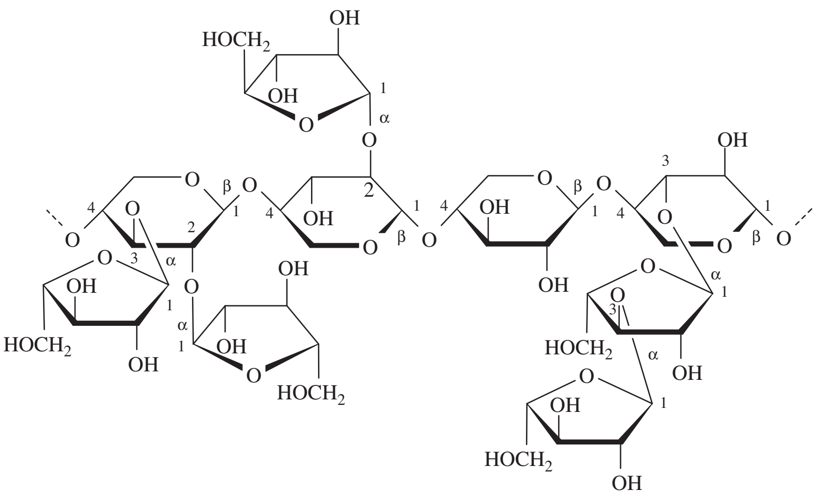
β-Glucans are particularly important in brewing technology. Their content in malting barley is 0.2–1% and varies according to a variety of climatic conditions, storage period and other factors. During mashing, water-soluble fractions of β-glucan are extracted, the accompanying proteins are hydrolysed by carboxypeptidases and initially insoluble substances are dissolved. β-Glucan molecules are hydrolysed by *endo*-β-(1→3), (1→4)-glucanase, *endo*-β-(1→3)-glucanase and β-glucosidase (laminarinase). The intermediates are soluble mixed linkage β-glucan dextrins and dextrins with β-(1→3) bonds. The final products are cellobiose, laminaribiose and glucose. The mashing temperature must not inactivate the enzymes hydrolysing the β-glucans, as native (undegraded) β-glucans can cause various problems in the production of beer. They prevent sedimentation of solid particles in the wort; beer filtration is complicated an unfiltrable haze or sediments can form in beer as a result of aggregation of β-glucan molecules among themselves or with other polymers, such as proteins and polyphenols.

β-Glucans of oats and barley reduce the bioavailability of feeds, which results in lower weight gain in poultry.

4.5.6.5.2 Heteroxylans

Structure

The main chain of heteroxylans consists of D-xylanopyranose units mutually linked by β-(1→4) bonds. The terminal unit is α-L-arabinofuranose. Most of xylose chain units are not substituted, although there is some substitution by α-L-arabinofuranose linked



α-L-Ara*f*
1
↓
3
····→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→····
2 2 3
↑ ↑ ↑
1 1 1
α-L-Ara*f* α-L-Ara*f* α-L-Ara*f*-(1→3)-α-L-Ara*f*

4-152, basic structure of arabinoxylans of cereals

by (1→3) bonds and less often by (1→2) bonds. Xylose is often substituted by two molecules of arabinofuranose (at C-2 and C-3), which forms short side chains connected by (1→2), (1→3) or (1→5) bonds (**4-152**). Some heteroxylans, such as heteroxylans from cereal brans, are acetylated on C-2 of the xylose residues. Heteroxylan molecules also contain sections in which xylose units are substituted twice, once or are unsubstituted. For example, wheat endosperm contains heteroxylans with xylose units substituted in position C-2 and C-3 (about 12–20%), xylose units (19–31%) substituted once (at C-3) and unsubstituted xylose units (55–69%). Rye heteroxylans contain chain segments exclusively substituted at C-2 and a smaller number of segments containing disubstituted xylose residues at C-2 and C-3.

With regard to the primary structure, these heteroxylans are called **arabinoxylans** and often also **pentosans**. The name is not very logical, because in addition to arabinose and xylose, these heteroxylans contain D-glucose and some other minor hexose building units such as D-galactose, D-glucuronic acid and its 4-*O*-methyl derivative, as well as some other sugars that are rarely found as terminal units in the side chains. The average xylose content is 52–60%, arabinose content is 36–46% and glucose content is 1.5–4.8%.

Arabinoxylans of various cereals differ not only in how the xylan chains are substituted, but also have different arabinose contents or ratios of these two sugars. The ratio of arabinose and xylose in wheat arabinoxylans varies from 0.50 to 0.71 and in less substituted rye arabinoxylans it ranges from 0.48 to 0.55. D-Glucuronic acid and its 4-*O*-methyl ether are found mainly in arabinoxylans of husked rice, rice hulls (husks), sorghum and maize brans. For example, these arabinoxylans, sometimes also referred to as **arabinoglucuronoxylans**, constitute about 4% of barley bran by weight.

A special feature is the presence of ferulic acid (its *cis*- and *trans* isomers in a ratio of about 1 : 1), which is 0.1–0.2% by weight of arabinoxylans. Ferulic acid is bound by an ester linkage at C-5 of the arabinose residue, which is connected to the C-3 of the xylose residue. In the secondary cell walls, ferulic acid forms ethers, C–C dimers and addition products with thiol residues of proteins, which play an important role in the development of cross-links between arabinoxylan molecules and other components of cell walls (Figure 4.18). Ferulic acid in lignified cells is bound to lignin. Proteins are also present (0.9–3.9% by weight) as in a number of other polysaccharides.

The average molecular weight of arabinoxylans of wheat ranges from about 220 to 260 kDa and of rye arabinoxylans from 520 to 770 kDa. The arabinoxylan molecules are relatively rigid long chains resembling cellulose chains. There is a clear link with the considerable viscosity of the dispersion and high ability to bind water.

Occurrence

Heteroxylans are the main polysaccharides of primary cell walls of vegetative parts of monocot plants and of lignified cells of monocot and dicot plants that are very important components of human diet. They occur in the stems of plants and in larger quantities in

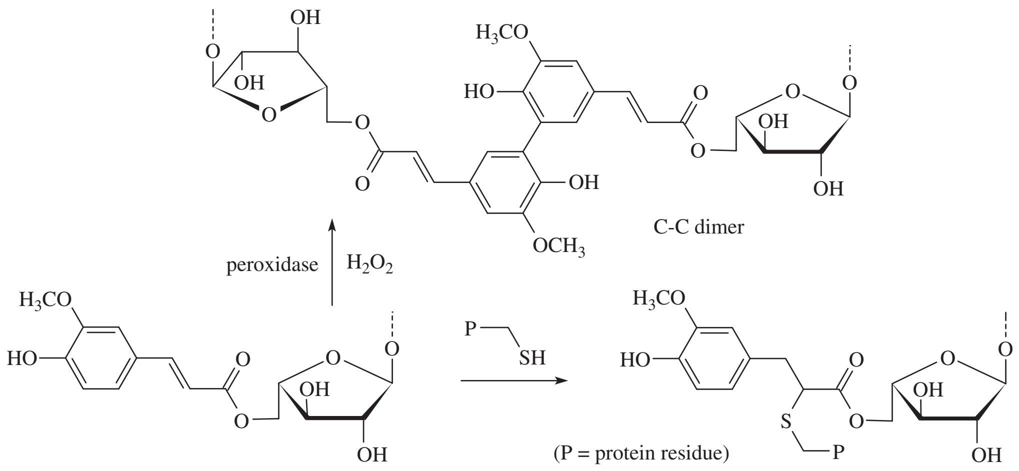


Figure 4.18 Reactions of ferulic acid residues in arabinoxylans.

maize ears that contain seeds (20–35%) and in wood mass (20–30% of dry matter).

In foods heteroxylans are mainly present in cereals, found in thin endosperm cell walls, the aleurone layer and lignified bran cells. The endosperm cell walls of most cereals contain 60–70% arabinoxylans, with 20% in barley and 40% in rice. Glumes (husks) of wheat grains contain about 64% heteroxylans. Wheat grains contain on average 1.4–2.1% of heteroxylans, of which 0.8–1.5% represent water soluble pentosans. Rice grains contain 7–8% heteroxylans.

Properties

Arabinoxylans have high water binding capacity (15–100 g of water per 1 g of dry matter). Some fractions are soluble in water and form extremely viscous dispersions. The differences in solubility depend on the degree of branching; therefore soluble molecules are more branched. In the presence of oxidising agents, arabinoxylans form soft and elastic gels. Ferulic acid bound in arabinoxylans plays a key role in their formation.

Soluble arabinoxylans are important components of wheat and particularly of rye flour. They have considerable influence on water uptake (hydration) by the flour and its distribution in the dough, the dough viscosity and its rheological properties. Other desirable baking properties of flour also depend on the presence of arabinoxylans (e.g. larger bread volume as a result of carbon dioxide retention, reduced rate of starch retrogradation connected with aging of bread and pastries, impact on desirable organoleptic properties of bread crust). Reaction products of water-soluble pentosans, especially reactions of ferulic acid with cysteine residues of gluten proteins, significantly contribute to the desirable rheological properties of dough, but water insoluble pentosans deteriorate the baking properties of flour. Like many other polysaccharides, arabinoxylans positively influence the composition of colon microflora.

4.5.6.6 Pectins

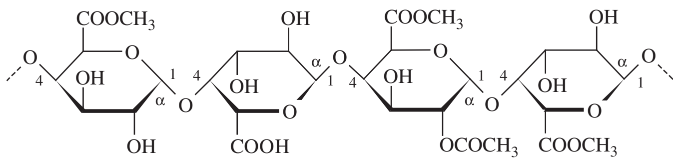
The general term **pectin**(s) or **pectic** (pectin) **substances** now refers to previously differentiated categories, which were polygalacturonates with higher amounts of methoxyl groups (**pectinic acids**), their salts **pectinans**, non-esterified polygalacturonates known as **pectic acids** and their salts **pectates** and the accompanying neutral polysaccharides (arabinans and arabinogalactans of different structures). Native, insoluble cell wall pectins associated with cellulose are known as **protopectins** (formerly pectoses). Enzymatic hydrolysis, by the enzyme complex protopectinase, converts protopectins into more or less soluble pectin substances with shorter chains, for example during fruit ripening.

Pectins are a group of highly polydisperse, complex, acidic polysaccharides of alternating chemical composition. They occur in all tissues of higher plants, except monocots, as part of the primary cell walls and intercellular space (Figure 4.17). Pectins are formed and stored mainly in the early stages of growth, when the area of the cell walls increases. The presence of pectins and their changes during growth, maturation, storage and processing have a major impact on the texture of fruits and vegetables.

4.5.6.6.1 Structure

A pectin molecule consists of three domains: homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II. These three polysaccharides are bound together by covalent bonds.

The basic structure of homogalacturonan consists of a linear chain composed of 25–100 units of D-galacturonic acid linked by α-(1→4) bonds. This polymer is often called **polygalacturonic acid**. Galacturonic acid units are, in various degrees (70% in average), esterified with methanol so they do not have any electrical charge, while the remaining units contain dissociated carboxyl group and are negatively charged. These carboxyl groups located in different chains can be cross-linked by calcium ions. Some α-D-galactopyranuronates or methyl α-D-galactopyranuronates are acetylated at positions C-2 or C-3 (**4-153**), which increases the polysaccharide hydrophobicity. Pectins of the goosefoot family of plants (Amaranthaceae, formerly Chenopodiaceae), such as spinach, also contain small amounts of ferulic acid linked by ester



···· →4)-α-D-Gal*p*A6Me-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A2Ac6Me-(1→4)-α-D-Gal*p*A6Me-(1→····

4-153, basic structure of homogalacturonans

bonds to neutral sugars (arabinose and galactose), similar to in arabinoxylans.

The degree of esterification (methylation) is defined as a percentage of esterified carboxyl groups. If the degree of methylation exceeds 50%, then the pectin is called **highly methoxylated** or **highly esterified** pectin. If the degree of methylation is lower than 50%, the pectin is called **low methoxylated** or **low esterified** pectin. The degree of acetylation is generally low, but sugar beet pectin contains a higher amount of acetyl groups (Table 4.32).

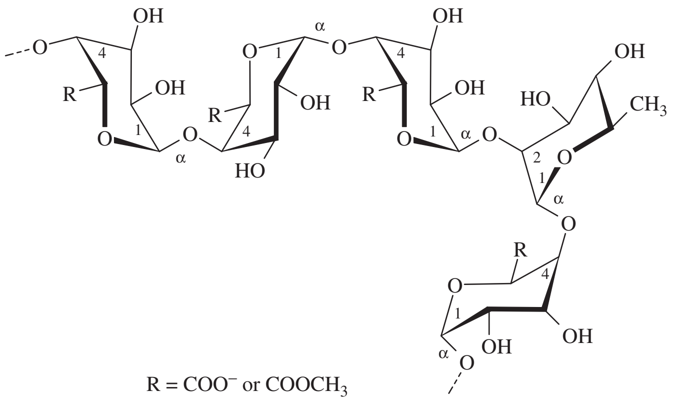
Rhamnogalacturonan I (degree of polymerisation is about 1000) contains chains of repeating units of α-D-galacturonic acid ($^{4}C_1$ conformers) with terminal α-L-rhamnopyranose linked by α-(1→2) bonds that correspond to the disaccharide residue α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-. The total content of rhamnose in pectin is 1–4%. Galacturonosyl and rhamnosyl residues are approximately in the ratio of 2 : 1 (**4-154**). Some units are methylated galacturonic acid molecules (4-*O*-methyl-D-galacturonic acid). About half of the rhamnosyl residues contain a galacturonic acid residue at C-4.

Molecules of rhamnogalacturonans I also contain a significant amount of branched side chains with a considerable number of arabinose and galactose units (**4-155**). These chains have the structure of **arabinans** and **arabinogalactants**. The side chains are attached to the main chain through a rhamnose molecule at C-4 or C-3, or less frequently through C-2 or C-3 of galacturonic acid. Pectins with type I arabinogalactans have been found in a number of fruits and vegetables. Apple pectins have 25–100% of rhamnosyl residues substituted by arabinose and galactose units, while carrot pectin substitution is 10–50%. About 32% of galacturonosyl residues of potato pectin and up to 75% of galacturonosyl residues of rapeseed pectin are branched.

Table 4.32 Content of galacturonic acid (GalA), degree of methylation (DM) and degree of acetylation (DA) of selected pectins.

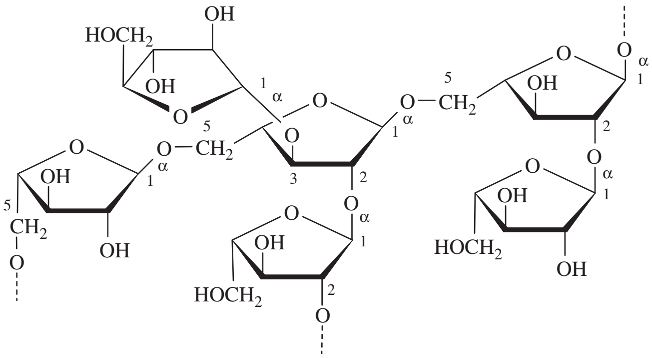
Pectin source	GalA (%)	DM (%)	DA (%)	Pectin source	GalA (%)	DM (%)	DA (%)
Apricots	64	57	8	Carrots	61	63	13
Peaches	90	79	4	Potatoes	40	53	15
Grapes	63	69	2	Sugar beet	65	62	35

Arabinans and galactoarabinans of rhamnogalacturonan I create the so-called hair regions of the pectin molecule, while smooth (unsubstituted) regions consisting of polygalacturonic acids in homogalacturonan are binding zones. Rhamnose is a sugar incompatible with the regular conformation of polygalacturonates; therefore its location in the chain determines the size of bonding zones that play a crucial role in the formation of pectin gels.



···→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→4)-α-D-Gal*p*A-(1→···

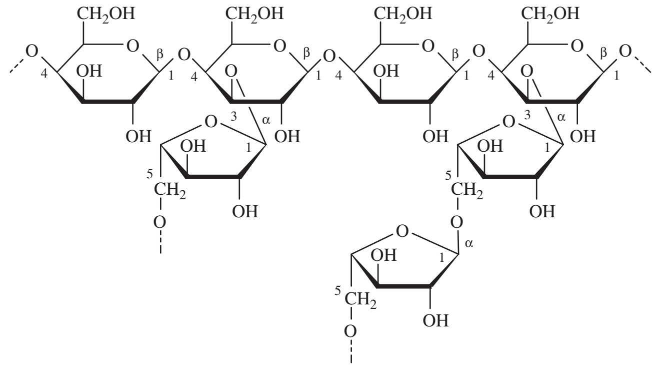
4-154, basic structure of rhamnogalacturonan I



α-L-Ara*f*
1
↓
3
····→5)-α-L-Ara*f*-(1→5)-α-L-Ara*f*-(1→5)-α-L-Ara*f*-(1→····
2 2
↑ ↑
1 1
····→2)-α-L-Ara*f* α-L-Ara*f*

4-155, general structure of arabinans in side chains of polygalacturonan I

Rhamnogalcturonan II is a low molecular weight polymer (degree of polymerisation is about 60 and relative molecular weight is about 4.8 kDa) with a chain consisting of α-D-galacturonic acids linked by α-(1→4) bonds. Different side chains composed of α- and β-D-galactopyranuronic acids, α- and β-D-rhamnopyranoses, α-D-galactopyranose, α-L-fucopyranose, α-L-arabinopyranose, β-L-arabinofuranose, α-D-xylopyranose and D-glucopyranuronic acid are connected to this main chain. The side chains also contain



····→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→····
3 ↑ 1 ····→5)-α-L-Ara*f*   3 ↑ 1 ····→5)-α-L-Ara*f*-(1→5)-α-L-Ara*f*

4-156, general structure of arabinogalactans in side chains of polygalacturonan I

four unusual sugar residues derived from 3-*C*-(hydroxymethyl)-β-D-erythrofuranose known as β-D-apiofuranose (β-D-Api*f*, 4-19), 3-*C*-carboxy-5-deoxy-β-L-xylofuranose (β-L-Ace*f*A, aceric acid, **4-83**), 3-deoxy-β-D-*lyxo*-hept-2-ulopyranaric acid (β-D-Dha*p*, **4-84**) and 3-deoxy-β-D-*manno*-oct-2-ulopyranosonic acid (β-D-Kdo*p*, from the previously used trivial name **keto****d**eoxy**o**ctonic acid; **4-85**). Xylose and fucose partly occur as 2-*O*-methyl ethers (**4-157**; anomeric configuration of the terminal L-galactopyranose is not known). The rings of rhamnogalacturonan II are furthermore cross-linked by boric acid, $B(OH)_3$ (borate ions), covalently bound to the *cis*-hydroxyl groups at positions C-2 and C-3 of the apiose molecules located in different chains of rhamnogalacturonan II. Therefore, this part of the pectin molecule is present predominantly as a dimer (see Section 6.3.18). The overall arrangement of pectin chains, indicating the binding zones responsible for the formation of gels, is shown schematically in Figure 4.19.

4.5.6.6.2 Occurrence

Pectins are found in virtually all fruits and vegetables. Their content is not high; in fruit flesh it varies around 1%. Higher amounts of pectin occur, for example, in apples, plums, damsons, currants, gooseberries, quinces and cranberries, while lower amounts are found in cherries, sour cherries, blueberries and elderberries (≤0.5%). Vegetables generally contain low levels of pectin; there the higher amounts are in tomatoes and carrots. Sugar beets also have a higher pectin level. The pectin content of some fruits and vegetables is given in Table 4.33.

4.5.6.6.3 Properties

Pectins are generally soluble in water and insoluble in most organic solvents. Water solubility decreases with increasing molecular weight and degree of esterification of the carboxyl groups (highly esterified pectins dissolve in hot water). Salts of polygalacturonic acids are generally more soluble than free acids (salts with monovalent ions are more soluble than calcium salts; salts of low esterified pectins are soluble in cold water).

β-L-Ara*f* 1 ↓ 4 β-D-Dha*p* 2 ↓ 3   α-L-Rha*p* 1 ↓ 5 α-D-Kdo*p* 2 ↓ 3
→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→····
2 ↑ 1 β-D-Api*f* 3 ↑ 1   2 ↑ 1 β-D-Api*f* 3 ↑ 1
α-D-Gal*p*A-(1→2)-β-L-Rha*p*-(3→1)-β-D-Gal*p*A β-L-Rha*p*
4 ↑ 1 β-D-Glc*p*A 2 ↑ 1 L-Gal*p*   3 ↑ 1 β-L-Ace*f*A 2 ↑ 1   α-L-Rha*p*-(2←1)-β-L-Ara*f* 1 ↓ 2
β-D-Gal*p*-(4←1)-α-L-Ara*p*-(3←1)-α-L-Rha*p*
2 ↑ 1 2-*O*-methyl-α-L-Fuc*p*

4-157, basic structure of rhamnogalacturonan II

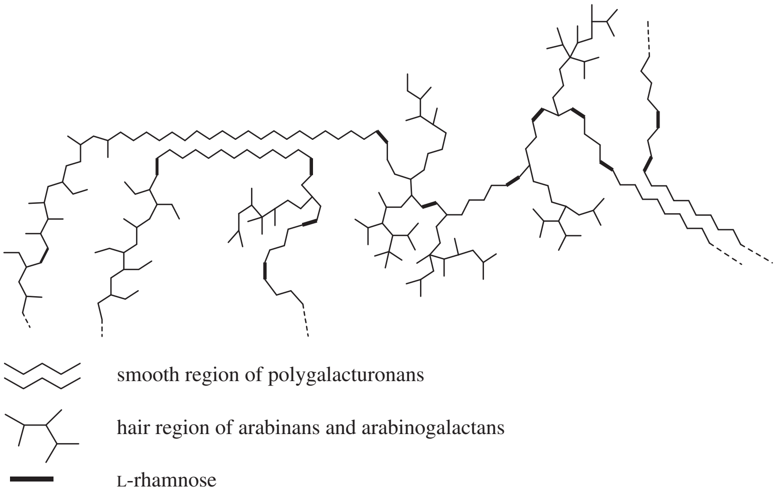


Figure 4.19 Pectin structure.

Table 4.33 Pectin content of fresh fruits and vegetables.

Pectin source	Pectin content (%)	Pectin source	Pectin content (%)
Apples	0.5–1.6	Grapes	0.1–0.9
Pears	0.4–1.3	Bananas	0.7–1.2
Peaches	0.1–0.9	Pineapple	0.04–0.13
Strawberries	0.6–0.7	Carrots	0.2–0.5
Gooseberries	0.3–1.4	Tomatoes	0.2–0.6
Red and black currants	0.1–1.8	Beans	0.5
Oranges	0.6	Onions	0.5
Peels of oranges	3.5–5.5	Potatoes	0.4

Pectin dispersion has a relatively low viscosity and therefore pectin is not used as a thickener. The viscosity of highly esterified pectins is increased by adding saccharose, and that of low esterified pectins increases in the presence of Ca^{2+} ions. Both types of pectins form gels under convenient conditions.

Formation of gels

The mechanism of gel formation depends on the degree of pectin esterification. Highly esterified pectins form gels with sugar in acidic solutions. Sugar binds water, thus reducing the degree of hydration of the pectin. Acids suppress the dissociation of carboxylic acid groups. The higher the degree of esterification, the smaller the amount of acid necessary, therefore totally esterified pectins form gels with sugar only. Fast gelling pectins form gels at pH $\cong$ 3.3, slowly gelling pectins form gels at pH $\cong$ 2.8. These gels are thermoreversible. Low esterified pectins (<50% of esterified carboxyl groups) form gels only in the presence of Ca^{2+} ions. Gelatinisation depends on the temperature, pH, ionic strength and amount of added calcium. Under acidic conditions (pH $\cong$ 3.5), higher amounts of calcium ions are needed than in less acidic solutions. Gels of low esterified pectins are also thermoreversible.

Pectin molecules have a negative electrical charge in a neutral environment (p$K \cong$ 3.5), and therefore react with polymers carrying positive charges, such as proteins (in solutions of pH < pI). Under acidic conditions, pectin stabilises casein, which allows heat treatment of fermented dairy products. The interaction of pectin with plant proteins affects the consistency and texture of fruit products.

Firmer thermoreversible gels arise in the presence of sodium alginate in acidic solutions (pH < 3.5), in the presence of low amounts of sugar and in the absence of calcium ions. Mechanical properties of these mixed gels depend on the ratio of pectin to alginate, and the degree of pectin esterification. Alginates with higher guluronic acid content form stable gels in combination with pectin with a degree of esterification of about 70%.

Changes and reactions

Hydrolysis of pectin substances proceeds by the action of enzymes and in acidic or alkaline solutions. A number of native enzymes and enzymes produced by microorganisms are involved in the enzymatic hydrolysis of pectins of fruits and vegetables. Two groups of enzymes can be distinguished:

- **pectinesterases**
- **pectin depolymerases**.

Pectinesterases (pectinmethylesterases or pectinpectylhydrolases) catalyse the hydrolysis of methyl esters to low esterified pectins, also known as pectic acids (Figure 4.20). The resulting acids react with the naturally present divalent ions, which may

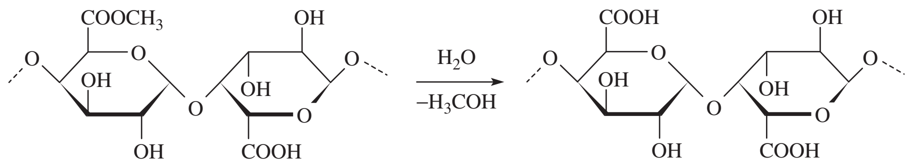


Figure 4.20 Reaction catalysed by pectinesterases.

lead to spontaneous gelling (in fruit juices) or hardening (in the processing of potatoes or cauliflower). Pectinesterases are present in various fruits and vegetables and are particularly active in cherries, citrus fruits, tomatoes and carrots. Also described are pectinacetylesterases splitting off acetyl groups in the smooth regions of pectin and rhamnogalacturonan acetylesterase that catalyses the same reaction in areas of branching. Together with rhamnogalacturonase, the latter enzyme also splits oligosaccharides containing rhamnose.

Enzymes hydrolysing glycosidic bonds (pectin depolymerases) are glycosidases and lyases. Glycosidases, termed polygalacturonases or poly-α-1,4-D-galacturonid glycanohydrolases, hydrolyse α-(1→4) glycosidic and ester bonds. Exoenzymes split off monomers from the end of the chain, while endoenzymes operate within the chain. The products of hydrolysis are either monomers or oligomers with variable chain lengths. Polymethylgalacturonases cleave highly esterified pectins; polygalacturonases break down completely (or almost completely) deesterified pectins (Figure 4.21). These enzymes are found in higher plants and microorganisms.

Pectate lyases (poly-α-1,4-D-galacturonid lyases) are also divided into exo- and endoenzymes. They degrade esterified or non-esterified pectins by a different mechanism to glycosidases, which is known as β-elimination (Figure 4.22). Pectate lyases are typical bacterial enzymes. Pectin lyases (poly-α-1,4-D-methoxygalacturonid lyases) cleave the glycosidic bond between esterified galacturonic acids by β-elimination (Figure 4.23). Only fungi produce pectin lyases.

Pectolytic enzymes usually act in combination. The enzyme exhibiting the activities of pectinesterase and polygalacturonase is sometimes referred to as pectinase. Industrially used pectinases are of bacterial origin, and often also have the activity of lyase, protease, cellulase and of other glycosidases.

Pectin is relatively stable under acidic conditions and its stability is highest in the pH range of 3–4. Hydrolysis of methoxyl and acetyl groups and hydrolysis of glycosidic bonds occurs in strongly acidic solutions. Free D-galacturonic acid can degrade to furan-2-carbaldehyde and other products. At elevated temperatures, hydrolysis of pectin occurs even in weakly acidic solutions (pH > 5); the polysaccharide chain is cleaved by β-elimination.

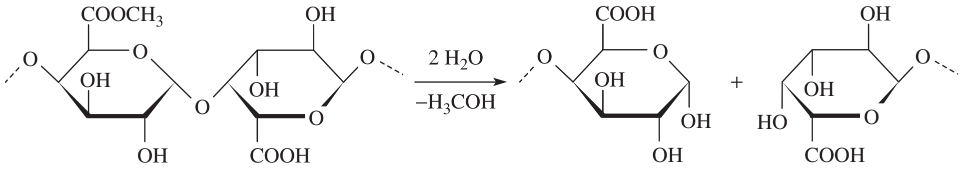


Figure 4.21 Reaction catalysed by polygalacturonases.

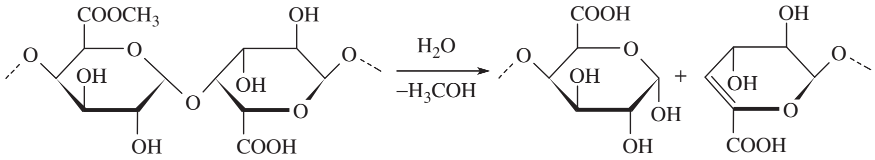


Figure 4.22 Reaction catalysed by pectate lyases.

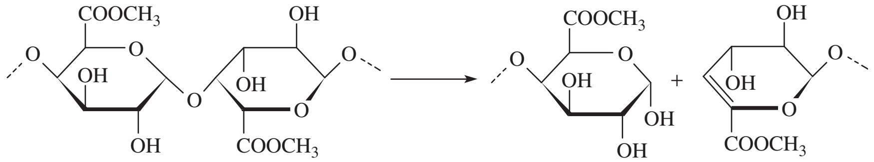


Figure 4.23 Reaction catalysed by pectin lyases.

This reaction is also important in alkaline solutions, where ester bonds are mainly hydrolysed and this reaction is accompanied by depolymerisation of the polysaccharide chains (β-elimination). The depolymerisation reaction occurs at the glycosidic bond of the non-reducing methoxylated galacturonic acid residue (Figures 4.22 and 4.23). Pectic acids are not depolymerised.

Treatment of a suspension of pectin in ethanol with ammonia yields **amidated pectin** that contains $-CONH_2$ functional groups. The degree of amidation is around 20%. Amidated pectin has a higher affinity for calcium ions in comparison with low esterified pectins.

4.5.6.6.4 Physiology and nutrition

Pectin belongs to the polysaccharides that form dietary fibre, which affects glucose metabolism and decreases the amount of cholesterol in the blood serum. Pectin, with a higher content of methoxyl groups, is more effective.

4.5.6.6.5 Significance and use

Insoluble pectic substances produce the hardness and strength of immature fruits and vegetables. During maturation, post-harvest storage and processing, these substances are subject to enzymatic and non-enzymatic degradation, which results in softening of fruits and the loss of the gelling ability of pectin. Pectins are released from complex polysaccharides that form the cell walls and this process continues after harvest during storage. Fruits containing active *endo*-polygalacturonases and pectinmethylesterases soften significantly and so quickly that this process often becomes an economic problem (e.g. in pears, cherries, kiwi and tomatoes). Softening is less pronounced in fruits containing only *exo*-polygalacturonases (apples, peaches). Changes during processing are suppressed by heat inactivation of pectolytic enzymes and by the addition of monovalent (softening) or bivalent (texture hardening) cations. Bivalent ions (such as Ca^{2+} ions) protect pectins against depolymerisation, which results in a firmer texture of the tissues; monovalent cations displace divalent ions, which have the opposite effect.

Pectins are also responsible for the consistency of sterilised fruits and vegetables, for the pressability of oilseeds, the filterability of fruit juices and the formation of hazes in fruit juices. Some manufacturing processes, for example in the canning industry, use pectolytic enzymes of microbial origin to increase the yield in the production of fruit juices and to maintain their clarity. Pectolytic preparations have also found use in the oenological industry, sugar industry and other sectors.

In industrial practice, pectins are mostly extracted from citrus fruit peels (their albedo), which contain about 20–40% of pectin. Another source of pectin is apple pomace, containing about 10–20% pectin. The isolation of pectin is based on extraction from an acidified aqueous slurry (pH 1.5–3) at temperatures of 60–100 °C. The extracts are then concentrated by evaporation or dried. Commercial pectin products are obtained by precipitation using metal ions that form insoluble salts with pectin (e.g. Al^{3+}), or by precipitation of pectin solutions with alcohols (ethanol or propan-2-ol).

4.5.6.7 Accompanying substances

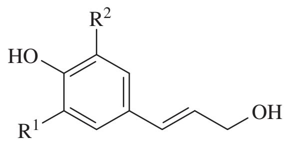
Cellulose and other structural polysaccharides of cell walls are associated with different polymeric non-sugar materials that fix and firm the cell walls and also form their outer hydrophobic layers, which are impermeable to water. In nutrition, they are classified as dietary fibre. According to their chemical composition, substances accompanying polysaccharides are classified as lignin, phenolic compounds (tannins), proteins or lipids.

4.5.6.7.1 Lignin

Occurrence

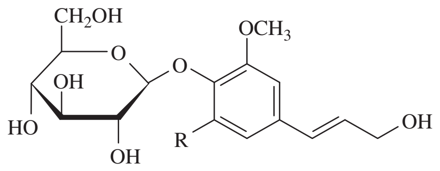
The structural polymer lignin is a major component of wood, where it makes up to about 25% of the biomass. Shelled nuts also have a similar amount of lignin. In lower amounts, lignin is a constituent of the dietary fibre of fruits, vegetables and cereals (Table 4.20). Primary cell walls are virtually free of lignin. A high content of lignin is found in lignified secondary cell walls, such as aleurone and subaleurone cells of cereals (in bran), which contain around 8% lignin. Lignin occurs in small amounts (tens to hundreds of mg/l) in spirits aged in oak barrels, where it is extracted from wood.

Lignin is a copolymer of phenylpropanoid units known as **monolignols**, which may include, according to the type of plant, 4-coumaryl alcohol, coniferyl alcohol (also known as ferulyl alcohol), 5-hydroxyconiferyl alcohol and sinapyl alcohol (**4-158**). These fenylpropanoid units (dominating structures are *trans*-isomers) are bound irregularly into three-dimensional structures by ether bonds (C–O–C) or bonds between two carbon atoms (C–C) and form the corresponding types of lignin (4-hydroxycoumaryl lignin, guaiacyl lignin, 5-hydroxyguaiacyl lignin and syringyl lignin). Also involved in the lignin biosynthesis are monolignol glucosides,



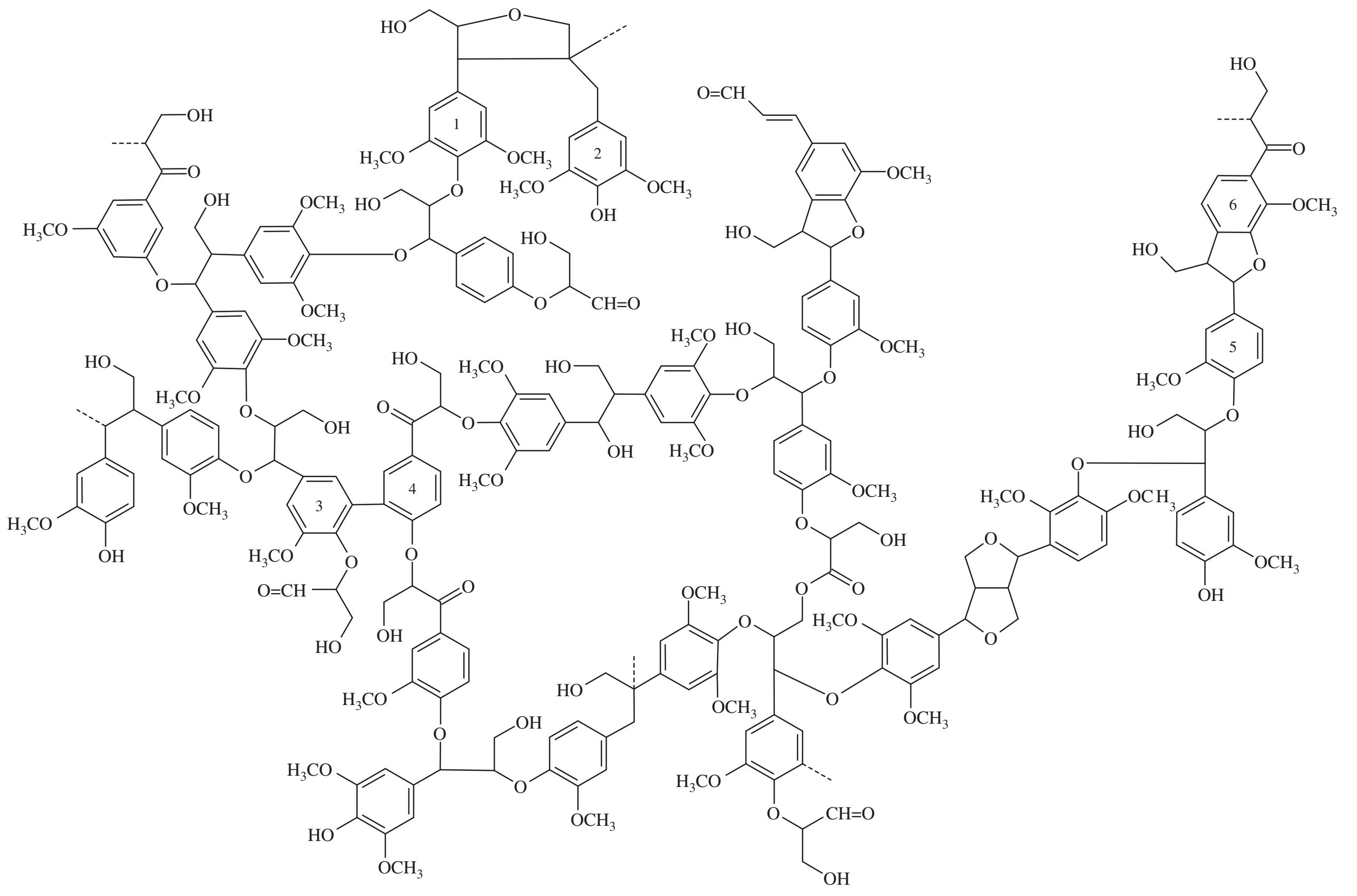
4-158, fenylpropanoid structural units of lignin

4-coumaryl alcohol, $R^1 = R^2 = H$
coniferyl alcohol (ferulyl alcohol), $R^1 = H$, $R^2 = OCH_3$
5-hydroxyconiferyl alcohol, $R^1 = OH$, $R^2 = OCH_3$
sinapyl alcohol, $R^1 = R^2 = OCH_3$



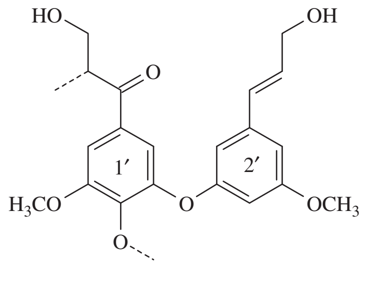
4-159, monolignol glucosides

coniferin, R = H
syringin, $R = OCH_3$

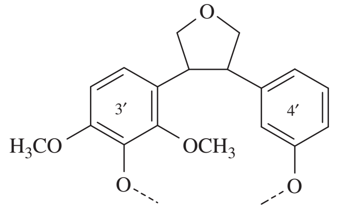


4-160, basic structure of lignin

aldehydes derived from monolignols and glucosides of these aldehydes. The most common monolignol glucosides are 4-*O*-β-D-glucopyranoside of (*E*)-coniferyl alcohol, known as coniferin, and 4-*O*-β-D-glucopyranoside of sinapyl alcohol, or syringin (**4-159**). The structure of birch wood lignin is given as an example (**4-160**). Dilignols 1′-2′ (**4-161**), 3′-4′ (**4-162**) and 5′-6′ (**4-163**) can replace the corresponding structural units 1-2, 3-4 and 5-6 with an incidence of 40, 50 and 10%.



4-161, alternative structures 1-2 in lignin (see **4-160**)



4-162, alternative structures 3-4 in lignin (see **4-160**)

Physiology and nutrition

The digestive system does not decompose lignin, and only cleaves the bonds between lignin and other polymers.

Use

During maturation of wines and spirits in oak barrels, lignin is decomposed to phenolic compounds that act as significant flavour-active components of these commodities. Phenolic components are also formed by pyrolysis of lignin in wood that is used in the process of smoking, and therefore are also found in smoked meat products and other smoked foods as well as in liquid smoke used for both food preservation and flavouring.

4.5.6.7.2 Other polymers

In addition to lignin, plants also contain structurally similar types of phenolic polymers, which are classified as **tannins** (see Section 8.3.6).

The best known structural protein of cell walls of plants is **extensin**, which is present in cells at levels of about 0.5–5%. Extensin is a glycoprotein with an unusual amino acid composition. It contains about 40% hydroxyproline and a large proportion of lysine and serine. Octasaccharides and decasaccharides composed of arabinose and galactose are bound through hydroxyamino acids, and phenolic compounds are bound through tyrosine molecules forming cross-links in the extensin molecule. Other structural proteins are present in smaller quantities, but may have an important function in building cell walls, as a connecting material with lignin.

Further components of dietary fibre are lipidic materials on the surface of cell walls that are composed of **waxes**, **cutin** (a cross-linked hydroxy fatty acid polyester) and **suberin** (a polyester composed of higher ω-hydroxy acids, α,ω-dicarboxylic acids, fatty acids and fatty alcohols, which also contains ferulic acid).

4.5.6.8 Plant gums and mucilages

Plant exudates, known as **vegetable gums**, are usually sticky juices flowing spontaneously from plant tissues as a result of different stress factors, especially the attack of microorganisms and injury. They eventually solidify in a solid gummy mass on contact with air. **Mucilages** are slimy secondary metabolites occurring in different parts (such as the fruits and seeds) of some plants. The basic chemical composition of gums and mucilages of some plants is shown in Table 4.34.

The polysaccharides of most important vegetable gums and mucilages are acidic polysaccharides. According to their primary structure, they may be divided into:

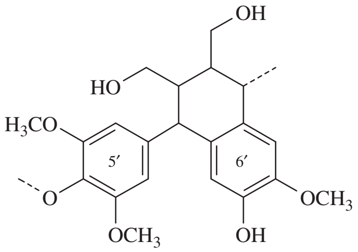
- substituted **arabinogalactans** (gum arabic, larch gum)
- mixed **arabinogalactans** and **glycanogalacturonans** (gum tragacanth)
- **glycanorhamnogalacturonans** (gum karaya)
- **glycanoglucuronomannoglycans** (ghatti gum)
- **glycanorhamnogalacturonans** (okra).

Vegetable gums and mucilages are highly hydrophilic, water-soluble polysaccharides. They are significantly polydisperse, branched and of very non-uniform structure. They are ranked among the hydrocolloids, but the low molecular weight fractions form true solutions. Dispersions or solutions are viscous, and in some cases gels may also arise. Plant gums often include non-starch storage polysaccharides of some seeds and tubers, such as guar, locust, tamarind and konjac gums.

4.5.6.8.1 Gum arabic

Occurrence

Gum arabic (also called acacia gum) is hardened exudate from acacia tree species, in particular from *Acacia senegal* and *Acacia seyal* (Fabaceae), growing mainly in Senegal, Nigeria and West African countries. There are over 100 different acacia gums, but the structural differences between them are small.

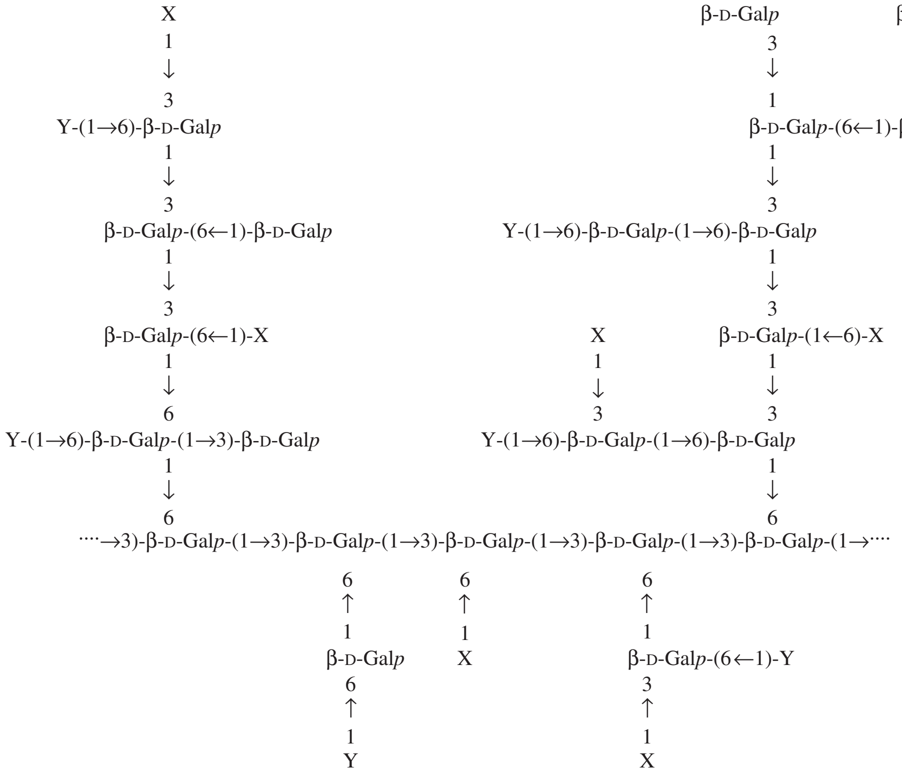


4-163, alternative structures 5-6 in lignin (see **4-160**)

Table 4.34 Basic monosaccharide composition of plant gums and mucilages (% w/w).

Gum or mucilage	Latin name of plant (family)	Neutral sugars							Uronic acids	
		Ara	Xyl	Rha	Fuc	Glc	Man	Gal	GlcA[a]	GalA
Arabic	*Acacia* spp. (Fabacaeae)	2–60	-	do 24	-	-	-	28–80	2–23	-
Tragacanth	*Astragalus* spp. (Fabaceae)	22	13	6	4	-	-	12	-	23
Karaya	*Sterculia* spp. (Malvaceae)	1	-	20–24	-	-	-	33–40	5	28
Ghatti	*Anogeissus latifolia* (Combretaceae)	32–41	2	7	2	-	7–8	27–42	6	-
Okra	*Abelmoschus esculentus* (Malvaceae)	-	-	30	-	-	-	30	-	30[b]

[a]Total amount of D-glucuronic acid (GlcA) and 4-*O*-methyl-D-glucuronic acid (GlcA4Me).
[b]Other sugars are D-glucose, D-mannose, L-arabinose and D-xylose, some sugars are partly acetylated.



X = α-L-Araf- or the sequence β-L-Arap-(1→3)-α-L-Araf-or α-D-Galp-(1→3) α-L-Araf-, Y = α-L-Rhap-(1→4)-β-D-GlcpA- or β-D-GlcpA4Me-

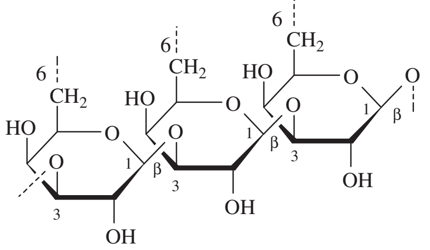
4-164, basic structure of gum arabic

Structure

Gum arabic is a substituted acid arabinogalactan. The basic building units are β-D-galactopyranose (the side chains also contain α-D-pyranose in small amounts), L-arabinose (α-furanose with small amounts of β-pyranose) and α-L-rhamnopyranose. β-D-Glucuronic and 4-*O*-methyl-β-D-glucuronic acids are present at lower levels. The gum from *A. senegal* contains around 44% galactose, 27% arabinose, 13% rhamnose and about 16% uronic acids (glucuronic and 4-*O*-methylglucuronic acids). In various types of acacia (*A. senegal*, *A. seyal*, *A. arabica*, *A. drepanolobium*, *A. karroo* and *A. sieberana*), the individual sugars are present over a relatively wide range of concentrations (Table 4.34).

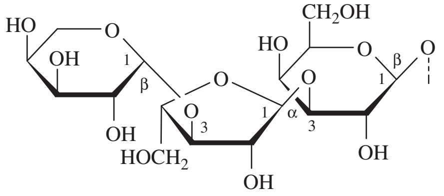
The main polysaccharide chain consists of β-D-galactopyranose units linked by (1→3) glycosidic bonds. Side chains, often repeatedly branched like amylopectin, occur in all residues of β-D-galactopyranoses of the main chain. β-D-Galactopyranose, linked to the main chain by β-(1→6) bonds and mutually by β-(1→6) and β-(1→3) bonds, predominates in the side chains. The abbreviated notation of a segment of gum arabic molecule originating from the plant *A. senegal* is given in formula **4-164**, which represents one of many possible structures. Some major segments of the molecule are shown in formulae **4-165** to **4-167**.

The relative molecular weight ranges from about 260 to 1200 kDa, but there are also polymers with molecular weights of several tens to 2 300 kDa. Molecules are spherical globules with a twisted main chain. Associated with the gum is a protein (1.5–3%), containing as the main amino acids hydroxyproline, serine and proline, bound by covalent bonds through arabinose in the side chain to hydroxyproline hydroxyl group.



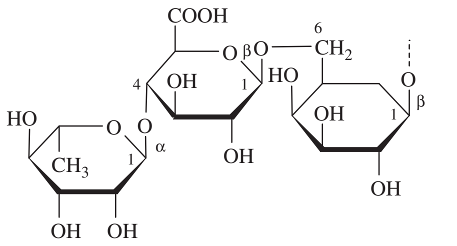
→ 3)-β-D-Galp-(1→3)-β-D-Galp-(1→3)-β-D-Galp-(1→

4-165, main chain fragment of gum Arabic



β-L-Arap-(1→3)-α-L-Araf-(1→3)-β-D-Galp-(1→

4-166, secondary chain fragment of gum arabic



α-L-Rhap-(1→4)-β-D-GlcpA-(1→6)-β-D-Galp-(1→

4-167, secondary chain fragment of gum arabic

Properties

The advantage of gum arabic is its very good solubility in water to dispersions of low viscosity. Dispersions containing 40% gum behave as Newtonian fluids, and dispersions of higher concentration of gum behave as pseudoplastic systems (due to aggregation of the molecules). Viscosity is, as in all other polyelectrolytes (acidic polysaccharides), strongly influenced by the pH of the medium and the presence of electrolytes (salts). Maximum viscosity is achieved at pH 4.5–8.0, but the presence of electrolytes decreases the viscosity. In the presence of sugar, concentrated solutions (approximately 40–50%) of gum arabic form soft gels; however, gels are not formed at commonly used concentrations.

Use

The ability of gum arabic to form concentrated dispersions (up to 50%), without significantly increasing their viscosity, is used for stabilising and emulsifying various food systems. The gum stabilises emulsions of oil-in-water as it is firmly adsorbed on oil droplets due to proteins bound to polysaccharides. In ice cream, gum arabic helps to maintain a smooth consistency (formation of small crystals), and in confectionery it prevents the crystallisation of sugars and wetting of icings. Gum arabic can also be combined well with other gums, starches, gelatine and sugars.

4.5.6.8.2 Larch gum

Occurrence

An industrial source of larch gum is the western larch (*Larix occidentalis*, Pinaceae), native to the mountains of western North America, whose wood contains up to 35% galactoarabans; these are obtained by extraction with hot water. Some other types of larch trees (*L. lyallii*, *L. sibirica*, *L. laricina* and *L. leptolepis*) have similar gums. Some species of pines, firs and other conifers may also be alternative sources.

Structure

Larch gum resembles the composition of gum arabic (arabinose content is usually 10–20%), but contains a lower amount of uronic acids. It consists of two fractions of nearly neutral arabinogalactans of different relative molecular weights (about 16 and 100 kDa). The main chain consists, as in gum arabic, of D-galactopyranose units linked by β-(1→3) bonds. The C-6 positions contain short side chains composed of arabinose and galactose (**4-168**).

Properties

Larch gum forms highly concentrated aqueous solutions (60%). It is stable in the pH range of 1.5 to 10.5, even in the presence of electrolytes.

Use

Larch gum is used in the food industry as a substitute for gum arabic. It is used also as a thickener and surfactant.

4.5.6.8.3 Tragacanth

Occurrence

The sources of tragacanth (also referred to as traganth, tragakanth, gum bassora or bassorin) are shrubs of the genus *Astragalus* (the common name milkvetch includes most species) belonging to the legume family Fabaceae. The most important plants are *A. gummifer*, *A. microcephalus* and *A. kurdicus*, growing in dry mountainous areas of Iran and Turkey.

Structure

Except for small amounts of starch and proteins (1.0–3.6%), tragacanth consists of two groups of polysaccharides. The first group is characterised by neutral arabinogalactans (so-called tragantin or tragacanthin), which represent about 60–70% of gum weight. Their structure is similar to that of larch gum. The main chain consists of units of D-galactopyranose linked by β-(1→4) bonds. Side chains

β-L-Arap-(1→3)-α-L-Araf   β-D-Galp-(1→6)-β-D-Galp   β-D-Galp-(1→6)-β-D-Galp
1   1   1
↓   ↓   ↓
6   4   6
····· →3)-β-D-Galp-(1→3)-β-D-Galp-(1→3)-β-D-Galp-(1→3)-β-D-Galp-(1→3)-β-D-Galp-(1→ ·····
6
↑
1
α-L-Araf-(1→3)-β-D-Galp

4-168, basic structure of larch gum

contain other units of D-galactopyranose linked by β-(1→6) bonds with terminal arabinose units attached to β-D-galactopyranose through (1→2), (1→3) and (1→5) bonds.

The second group of acidic polysaccharides of the pectin type (so-called tragacanthic acid or bassorin) consist of α-D-galacturonic acid (or its potassium salt) units linked by (1→4) bonds. Tragacanthic acid also contains small amounts of α-L-rhamnopyranose. Units of the main chain are either unsubstituted or substituted by β-D-xylanopyranose residues, or by short chains formed by xylose and β-D-galactopyranose that can be replaced by α-L-fucose (**4-169**). The side chain also contains D-glucuronic acid. Some sugars are acetylated and α-D-galacturonic acid is methylated analogously to pectin. The average molecular weight is 840 kDa.

Properties

The neutral fraction of tragacanth is soluble in water; acidic fractions only swell and form a thick slime or gel in the presence of Ca^{2+} ions. The viscosity of sols is high even at low concentrations, where the dispersion behaves as a Newtonian fluid (in the concentration range of from 0.3 to 0.5%). The pH value of solutions has little effect on viscosity and viscosity does not change under acidic conditions and even at pH 7–10. An important feature of tragacanth is its resistance to hydrolysis and mechanical stress.

Use

Tragacanth is used as a thickening agent, emulsifier and stabiliser in salad dressings, ice creams, pastry fillings and other products.

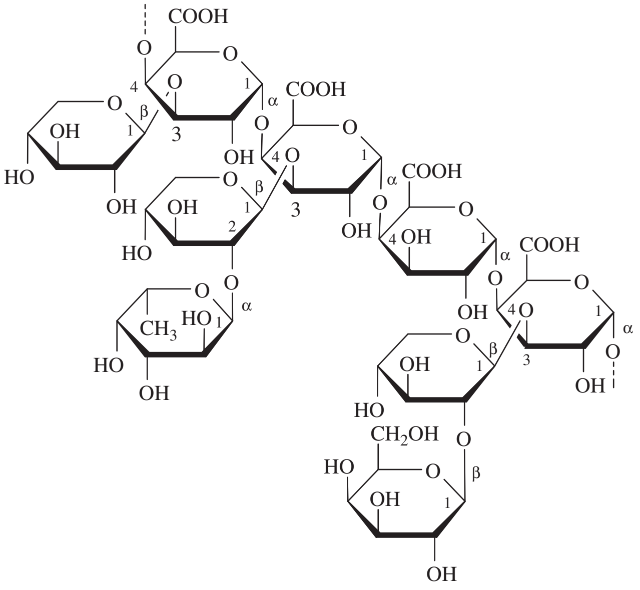
4.5.6.8.4 Gum karaya

Occurrence

Gum karaya (sometimes referred to as Sterculia gum) is a bark exudate of trees of the genus *Sterculia* (Sterculiaceae), mainly of *S. urens*, growing on the plateaus of central and northern India. Similar properties are found in some other species of the genus Sterculia, for example *S. setigera*, *S. caudata* and *S. villosa* or some species of the genus *Cochlospermum* of the Cochlospermaceae family.

Structure

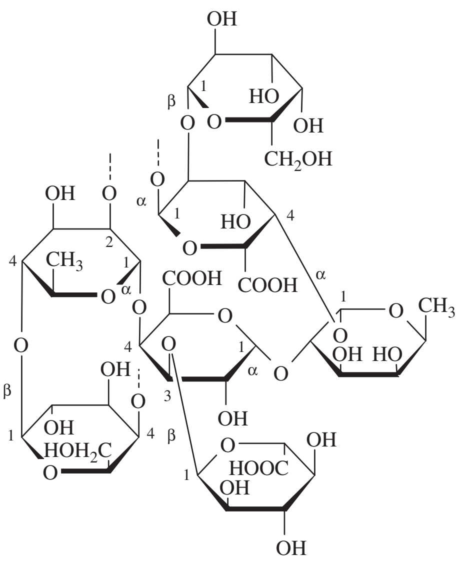
Gum karaya is glycanorhamnogalacturonan. The main polysaccharide chain consists of alternating units of L-rhamnopyranose and D-galacturonic acid, which are connected by α-(1→4) and α-(1→2) glycosidic bonds. Units of the main chain are substituted by β-D-galactopyranose and β-D-glucuronic acid or remain unsubstituted. The end units are rhamnose, galactose and glucuronic acid; some units are partially acetylated (**4-170**). Gum karaya contains



··· →4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→4)-α-D-Gal*p*A-(1→ ···

3	3	3
↑	↑	↑
1	1	1
β-D-Xyl*p*	β-D-Xyl*p*	β-D-Xyl*p*
	2	2
	↑	↑
	1	1
	α-L-Fuc*p*	β-D-Gal*p*

4-169, basic structure of tragacanth acidic component



···→2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→···

(branches: at position 4 of the first Rhap: β-D-Galp-(1→4), itself substituted at position 4 (4 ↑); at position 3 of the first GalpA: β-D-GlcpA-(1→3); at position 2 of the second GalpA: β-D-Galp-(1→2))

4 ↑ 1 β-D-Galp 4 ↑   3 ↑ 1 β-D-GlcpA   2 ↑ 1 β-D-Galp

4-170, basic structure of gum karaya

calcium and magnesium ions bound to the carboxylic acid groups of glycuronic acids.

Properties

Gum karaya is very slightly soluble. At very low concentrations (<0.02% in cold water and 0.06% in hot water) it forms true solutions. Dispersions of a concentration of 0.3–0.5% show behaviour of newtonian liquids as well as dispersions of tragacanth, guar and locust gums. At higher concentrations (up to 5%), gum karaya forms viscous colloidal dispersions, which behave as non-Newtonian liquids.

Use

Gum karaya is used as a thickener for soups, sauces, ketchups and mayonnaise, as a substance that increases water binding capacity in processed cheese and meat products and for stabilisation of foams produced from proteins (egg white whipped cream or whipped cream). It is also used in the pharmaceutical industry.

4.5.6.8.5 Ghatti gum

Occurrence

Ghatti gum (also known as gum ghati or Indian gum) is an exudate of *Anogeissus latifolia* trees (Combretaceae) growing in arid regions of India, Ceylon and Africa (Ghana). The gum of *A. leiocarpus* has similar properties. Ghatti gum is not very significant commercially.

Structure

The molecule of glycanoglucuronomannoglycan (formerly called ghattic acid) is formed by periodically repeating residues of α-D-mannose and β-D-glucuronic acid, which are linked in the main chain through (1→4) and (1→2) bonds. The side chain contains mannose, to which is linked α-L-arabinofuranose by (1→6) bonds, and by (1→3) bonds β-L-arabinopyranose to which β-D-glucuronic acid or β-D-galactopyranose is bound (**4-171**). The average molecular weight is about 12 kDa. The gum of the *A. leiocarpus* tree contains two mutually different polysaccharides of similar composition. The minor component is leiocarpan A and the major component is leiocarpan B.

X 1 ↓ 6   X 1 ↓ 6

··· →4)-β-D-GlcpA-(1→2)-α-D-Manp-(1→4)-β-D-GlcpA-(1→2)-α-D-Manp-(1→ ···

3 ↑ 1 β-L-Arap 3 ↑ 1 Y   3 ↑ 1 β-L-Arap 3 ↑ 1 Y

X = α-L-Araf or α-L-Araf-(1→2, 3 or 5)-α-L-Araf-(1→···

Y = β-D-GlcpA-(1→6)- or β-D-Galp-(1→6)-[β-D-Galp]$_n$-(l→···

3 ↑ X

4-171, basic structure of ghatti gum

Properties

The functional properties of ghatti gum are more similar to those of gum arabic than to viscous gums tragacanth and karaya. Ghatti gum has low solubility in water and does not form a gel. At concentrations lower than 5% it forms true solutions, and at higher concentrations viscous colloidal dispersions result.

Use

Ghatti gum, thanks to its higher viscosity, has found use as a stabiliser of emulsions and suspensions.

4.5.6.8.6 Okra and other plant mucilages

Plant mucilages are used in many eastern Mediterranean, West Asian and African countries, and are popular in Indian, Pakistani, Chinese, Malaysian, Japanese, Caribbean and many other cuisines as ingredients in soups and sauces, to which they give their characteristic mucilaginous consistency. The main representative of this group of polysaccharides is **glycanorhamnogalacturonan** that

comes from unripe fruits of edible hibiscus (*Abelmoschus esculentus*) of the mallow family (Malvaceae), which is known as **okra** (also bamie or gombo), and commonly known in many English-speaking countries as lady's fingers.

The basic structural unit of the main okra chain is acidic disaccharide →4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→. Side chains contain the disaccharide D-galactobiose, β-D-Gal*p*-(1→4)-β-D-Gal*p*-(1→, that is linked by (1→4) bound to about one half of the α-L-rhamnopyranose residues.

Okra polysaccharides are already viscous in diluted solutions (0.5%), and the viscosity increases rapidly with increasing concentration. Heating increases their solubility, but after some time leads to irreversible loss of viscosity caused by hydrolysis. The maximum viscosity is achieved at pH 6–9. Okra mucilage is a typical polyelectrolyte. It has hypoglycaemic effects.

Other acid mucilages of composition similar to that of okra (glycanorhamnogalacturonans) are **junsai**, originating from the leaves and stems of plant *Brasenia schreberi* (Cabombaceae), **baobab mucilage**, obtained from the leaves of baobab *Adansonia digitata* (Malvaceae) and **ruredzo** from the plant *Dicerocaryum zanguebarium* (Pedaliaceae). Slimy substances are produced by many other plants, but these mucilages are usually important locally and are not used commercially.

4.5.7 Seaweed polysaccharides

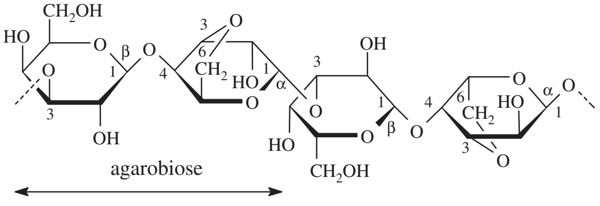
The most important representatives of this group of polysaccharides are:

- **agar**
- **carrageenans** and **furcellaran**
- **alginates** (alginic acid salts).

4.5.7.1 Agar

Agar forms an intracellular matrix of a number of species of red seaweed (Rhodophyceae) that plays a similar role in algae as cellulose does in higher plants. Algae, which are a source of agar (agarophytes), come mainly from the Gelidaceae, Gracilariaceae and Pterocladiaceae families, which grow on the coast of Portugal, South Africa, India, Japan, Mexico, Chile and New Zealand. Agarophytes are usually wild algae and are only grown in marine farms in Chile. Agar is obtained from algae usually by extraction with hot neutral, acidic or alkaline water (at a temperature higher than the melting point of the agar gel). Gels obtained from extracts are dried. Alkaline solutions cause partial hydrolysis of the sulfate groups, which results in modified agar properties.

The building units of linear polysaccharides agars are β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose alternately linked by glycosidic (1→3) and (1→4) bonds. The basic neutral polysaccharide is often still called **agarose** (by analogy with starch amylose). Its building block is disaccharide agarobiose, 4-*O*-β-D-galactopyranosyl-3,6-anhydro-α-L-galactopyranose (**4-172**). The structure of agars is in fact much more complex. Some polysaccharide fractions contain α-L-galactose instead of 3,6-anhydro-α-L-galactose. Hydroxyl groups at C-6 of β-D-galactose or at C-2 of α-L-galactose can be partially methylated. Hydroxyl groups at C-4 of β-D-galactose and at C-6 of α-L-galactose are esterified with sulfuric acid (the SO_4^{2-} ion concentration is up to about 7% w/w). These fractions also contain bound pyruvic acid (as acetal in positions C-4 and C-6 of β-D-galactose) in 4,6-*O*-(1′-carboxyethylidene)-D-galactose, the concentration of which is 0.02–1%. These fractions also contain about 1% of D-galacturonic acid. Previously, these acidic agar fractions were known as **agaropectin** (by analogy with amylopectin). According to recent studies, up to 18% of total sugars in some agars represent D-xylopyranose molecules substituted in some units of the main chain.



·· →3)-β-D-Gal*p*-(1→4)-α-L-Gal*p*3,6An-(1→3)-β-D-Gal*p*-(1→4)-α-L-Gal*p*3,6An-(1→ ··

4-172, agarose

Agars are largely polydisperse polysaccharides whose molecular weight ranges from 80 to 420 kDa. These molecules, similarly to other algal polysaccharides, form organised structures (double right-handed helices).

Agars are soluble in hot water at 85 °C and at higher temperatures and the dispersion yields gel by cooling. Commonly used concentrations range from 0.5 to 2.0%, but gels are formed even in 0.04% solutions. The sol–gel transition and the reverse process show a hysteresis. The melting point temperature of the gel (at temperatures up to 95 °C) is higher than the temperature at which the gel forms. During aging, agar gels are subject to syneresis. Their resistance to deformation is improved when combined with vegetable gums, such as locust gum. Agars are polysaccharides of low acidity and, unlike carrageenans, the gel formation does not require the presence of neutralising cations.

The use of agar in food production is based on its ability to bind water and form thermoreversible gels. Its gelling properties depend on the proportion of the agarose fraction present. Owing to the high melting point of gels, agar is mainly used in bakery products, as well as in the production of jams and jellies, confectionery products, dairy products, meat, fish and poultry products and beverages. In some Asian countries (e.g. in Japan), agar is used not only as a food additive, but also as a separate food (in various types of flavoured gels or in edible packagings).

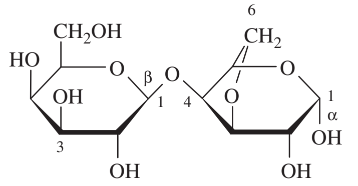
4.5.7.2 Carrageenan

Carrageenan is an extract from red seaweed (Rhodophyceae), especially of the genera *Chondrus* and *Gigartina*. Carrageenans differ in their structure, which is largely related to their origin.

Seaweed algae of the genus *Euchema* (*E. cottonii* and *E. spinosum*) are fibrous shrubs of about 0.5 m in height, which grow on coral reefs along the Philippines, Indonesia and other tropical regions of the Pacific Ocean. They are also grown on marine farms. The algae *Chondrus crispus* (also known as Irish moss) are small, dark red shrubs growing to a height of about 0.1 m along the coast of the North Atlantic, particularly in Canada, the British Isles and France. Algae of the genus *Gigartina* grow to a height of up to 5 m in the cool coastal waters of South America (Chile).

Carrageenans are mainly extracted as sodium salts using alkaline hot water (Na_2CO_3 or NaOH solutions). On acidification (HCl) carrageenans are obtained. The final materials are produced by drying or by precipitation with solvents (such as propan-2-ol).

Carrageenans are linear polysaccharides with a structure similar to the structure of agars but, unlike agars, the structural unit is only D-galactopyranose and not L-galactopyranose. The structure is a repetitive sequence of β-D-galactopyranose and 3,6-anhydro-α-D-galactopyranose, a disaccharide, known as carabiose (**4-173**). In fact, the primary structures of carrageenans are much more complex. They can be expressed by notation ⋯ → 3)-β-A-(1→4)-α-B-(1→3)-β-A-(1→4)-)-α-B-(1→ ⋯ , where A and B are units of D-galactose; its derivatives are listed in Table 4.35. For comparison, the structure of polysaccharide agarose is also given.



β-D-Gal*p*-(1→4)-α-D-Gal*p*3,6An

4-173, carabiose

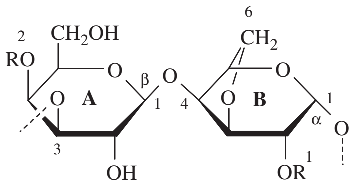
Sequences of at least eight types of monomers in the carrageenan molecules are known. They are indicated with lower case Greek letters: β (beta), θ (theta), ι (iota), κ (kappa), λ (lambda), μ (mu), ν (nu) and ξ (xi). The same names are used for the individual carrageenans if these sequences predominate in their primary structures. In the food industry, attention is devoted to just three dominant species, known as ι-carrageenan (its precursor is ν-carrageenan), κ-carrageenan (its precursor is μ-carrageenan) and λ-carrageenan. These three carrageenans are highlighted in bold in Table 4.35.

Carrageenans obtained from algae are predominantly complex mixtures of polysaccharides. They can be fracionated by precipitation with potassium salts (a KCl solution of concentration 0.25 mol/l), which separates the two main components, insoluble κ-carrageenan and soluble λ-carrageenan. The potassium salt of κ-carrageenan is insoluble in cold water, whereas the potassium salt of ι-carrageenan is slightly soluble in cold water, but soluble at temperatures above 60 °C, and λ-carrageenan is soluble in the form of any salt. Virtually pure carrageenans can also be obtained from certain species of algae; for example, ι-carrageenan from *Euchema cottonii* algae, κ-carrageenan from *E. spinosum algae* and λ- carrageenan from *Chondrus crispus* algae.

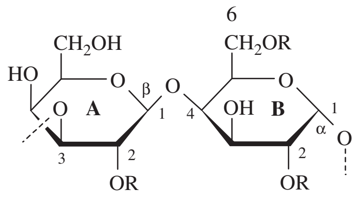
Table 4.35 Basic structure of carrageenans.

Polysaccharide	Building unit A	Building unit B
Agarose	**β-D-Galactose**	**3,6-Anhydro-α-L-galactose**
β-Carrageenan	β-D-Galactose	3,6-Anhydro-α-D-galactose
κ-Carrageenan	**β-D-Galactose-4-sulfate**	**3,6-Anhydro-α-D-galactose**
ι-Carrageenan	**β-D-Galactose-4-sulfate**	**3,6-Anhydro-α-D-galactose-2-sulfate**
μ-Carrageenan	β-D-Galactose-4-sulfate	α-D-Galactose-6-sulfate
θ-Carrageenan	β-D-Galactose-2-sulfate	3,6-Anhydro-α-D-galactose-2-sulfate
ξ-Carrageenan	β-D-Galactose-2-sulfate	α-D-Galactose-2-disulfate
ν-Carrageenan	β-D-Galactose-4-sulfate	α-D-Galactose-2,6-disulfate
λ-Carrageenan	**β-D-Galactose-2-sulfate**	**α-D-Galactose-2,6-disulfate**

The structure of the repeating units A and B in κ-carrageenan ($R^1 = H$, $R^2 = SO_3^-$), ι-carrageenan ($R^1 = R^2 = SO_3^-$) and λ-carrageenan ($R = SO_3^-$) are provided in formulae **4-174** and **4-175**, respectively. In addition to sulfate groups, and similarly to agar, carrageenan macromolecules also contain other functional groups (methoxyl groups or pyruvic acid bound as acetal).



4-174, basic structure of κ- and ι-carrageenans



4-175, basic structure of λ-carrageenan

The average molecular weights of all three very polydisperse polymers ranges from 100 to 1 000 kDa. In the sequence shown in Table 4.35, the sulfate group contents of the polysaccharides

increase, which is associated with conformations of the molecules and the carrageenan properties. In the same way as agar molecules, molecules of κ-carrageenan and ι-carrageenan form double helices, but molecules of λ-carrageenan occur in a zigzag conformation due to sulfuric acid bound at C-2 and C-6 of the B unit (disulfate is primarily found in the $^4C_1$ conformation, but the formation of helical structures needs a $^1C_4$ conformation of the 3,6-anhydro derivative). Gelling properties can be improved by intramolecular substitution of sulfate group at C-6 of the B units in alkaline solution, which yields 3,6-anhydrogalactose-2-sulfate (θ-carrageenan).

Carrageenans are anionic hydrophilic colloids. Solubility in water depends on the type of carrageenan, ions present, temperature and pH. The ratio of hydrophilic hydroxyl and sulfate groups and hydrophobic 3,6-anhydro-D-galactose residues also affects the solubility. Highly sulfated λ-carrageenan is very soluble and forms viscous dispersions (but does not form gels). κ-Carrageenan containing higher amounts of hydrophobic and less hydrophilic groups is less soluble, and the solubility of ι-carrageenan is between the solubility of κ-carrageenan and λ-carrageenan. Carrageenans are stable in solutions of pH 5–10, but in more acidic solutions (pH < 4) they are hydrolysed and the viscosity of dispersions decreases.

An important feature is the formation of gels. They are formed, like agar gels, by cooling dispersions of κ- or ι-carrageenans of even 0.5%, where intermolecular associations of double helices lead to the formation of superhelical structures (Figure 7.28). κ-Carrageenan forms a firm, brittle gel that undergoes syneresis, ι-carrageenan provides flexible and cohesive thixotropic gels, where syneresis does not occur. The formation of strong but brittle gels requires the presence of neutralising ions (e.g. potassium or ammonium ions, but not sodium ions in κ-carrageenan or calcium ions in ι-carrageenan).

An important property is the ability of carrageenans to form complexes with milk proteins (caseins). They can also be combined with modified starches.

Commercial carrageenan is a mixture of all three types of carrageenans in which κ-carrageenan (gelling) mostly prevails over λ-carrageenan (non-gelling) in a ratio of about 3 : 2. Carrageenan is used as a thickener, gelling agent, stabiliser and emulsifier in the production of dairy desserts, milk drinks and ice creams, and in the manufacture of canned meat. It also finds applications in cosmetics, for stabilisation of industrial suspensions and in the production of various pigments.

4.5.7.3 Furcellaran

Furcellaran is obtained mainly from red algae of the genus *Furcellaria* (*F. lumbricalis* and *F. fastigiata*). Sometimes it is also known as Danish agar, because its source is predominantly along the coast of Denmark. Furcellaran is a sulfated polysaccharide consisting of D-galactose units (46–53%), 3,6-anhydro-D-galactose (30–33%) and their sulfates (16–20%). Its structure and properties are similar to κ-carrageenan, and it is therefore often considered one of the carrageenans. The difference between furcellaran and κ-carrageenan lies in the fact that κ-carrageenan has a sulfate group linked to every second sugar unit, while in furcellaran it is to every third unit.

Furcellaran is soluble in warm water and forms soft, flexible and thermoreversible gels. The addition of sugar positively affects the strength of the gel. Furcellaran forms very strong gels in the presence of K^+ and $NH_4{}^+$ ions the same as κ-carrageenan, the presence of Ca^{2+} ions has less effect and with Na^+ ions it does not produce gels. It is used in the production of milk puddings and desserts.

4.5.7.4 Algin

4.5.7.4.1 Occurrence

Algin is the name used to cover **alginic acid** and its salts, **alginates**. Algin is an intercellular matrix (a gel containing ions of Na, Ca, Mg, Sr and Ba) in the brown seaweed family Phaeophyceae growing off the coast of the North Atlantic, especially in the United States, Norway, France and Great Britain. The main industrial sources are algae *Macrocystis pyrifera*, *Laminaria hyperborea* and algae of the genus *Ascophyllum* and *Sarrgasum*. Alginate makes up about 40% of dry matter of algae.[8]

Algin, like agar and carrageenan, is obtained as the sodium salt (alginate) by extraction of algae with alkaline aqueous solutions (NaOH or Na_2CO_3). The extract is precipitated as calcium salts by adding $CaCl_2$ or as alginic acid by acidification using HCl. The calcium salt is converted into alginic acid, from which the final commercial product (sodium salt) is obtained by neutralisation with Na_2CO_3. A significant portion of native polysaccharides are hydrolysed during the isolation of alginates.

4.5.7.4.2 Structure

Alginates are linear copolymers of non-branched β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by (1→4) glycosidic bonds (**4-176**). Residues of mannuronic acid are in the $^4C_1$ conformation, while residues of guluronic acid are in the $^1C_4$ conformation. The chain contains alternating sections of different

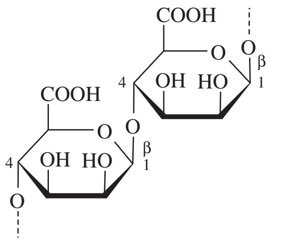
[8]A range of extracellular bacterial polysaccharides of the genera *Azotobacter* (*A. vinelandii* and *A. crococcum*) and *Pseudomonas* (*P. aeruginosa*, *P. putida*, *P. mendocina*, *P. Fluorescens* and *P. syringae*), known as bacterial alginates, have similar compositions and properties. They are almost exclusively polymannuronans. Some bacteria are considered as a future potential industrial source of bacterial alginate, which could replace production from algae.

M−M−M−M−G−M−G−G−G−G−G−M−G−M−G−G−G−G−G−G−G−M−M−G−M−G−M−G−G−M

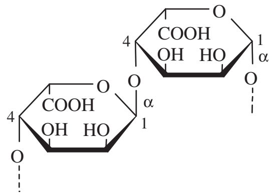
section M ⟷ section G ⟷ section G ⟷ section M-G ⟷

4-176, basic ctructuru of alginates (M = β-D-mannuronic acid, G = α-L-guluronic acid)

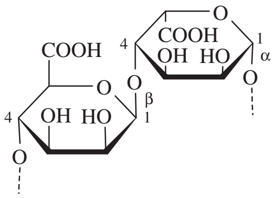
lengths containing only molecules M, sections formed exclusively of molecules G and mixed sections G-M. Representation and alternation of both components is particularly variable, depending mainly on the origin of the alginate. The mannuronic acid content is usually 22–90%, and that of guluronic acid varies between 10 and 78%. The structures of alginate sections M-M, G-G and M-G are shown in formulae **4-177** to **4-179**.



4-177, structure of alginate section M-M



4-178, structure of alginate section G-G



4-179, structure of alginate section M-G

4.5.7.4.3 Properties

Alginates of alkali metals, ammonium salts, amine salts and magnesium salts are soluble, while calcium salts are insoluble. Solubility is affected by pH, ionic strength and ion type. Alginic acid is precipitated in acidic solution and slow acidification gives a precipitate rather than a gel. Solutions of alkali metal alginates are highly viscous and behave as pseudoplastic systems. Viscosity is strongly dependent on ionic strength (they are polyelectrolytes); as the ionic strength increases the viscosity decreases. By heating and in the presence of reducing agents (such as ascorbic acid), the polymers are degraded and viscosity decreases.

An important property of alginates is the formation of gels and heat-resistant films that easily form on addition of calcium ions to sodium alginate dispersions. In the binding of calcium ions, not only do electrostatic forces participate, but their chelation is also involved. The binding zones are the G sections that contain at least four units of guluronic acid. This creates a structure called the egg-box structure (Figure 7.31), as in pectin. Depending on the guluronic acid concentration (the amount in the binding zones), the formed gels have different properties. The relative molecular weight of alginates is 32–200 kDa and the chains (spiral to almost linear) contain 180–930 sugar units.

4.5.7.4.4 Use

Alginates are used, in concentrations ranging from 0.25 to 0.5%, as thickeners, stabilisers and emulsifiers to improve the consistency of bread, sauces, dressings, ice creams, fruit juices and many other foods. Their gelling properties are employed in the production of fruit and dessert jellies, puddings and reconstituted fruit prepared from fruit pulp. With highly esterified pectins thermoreversible gels are formed. Alginates also readily react with protonised amino acid residues in proteins to form precipitates, and may therefore also be used to remove proteins, for example from beer. Besides the native alginates, alginates modified by propylene oxide (propylene glycol alginates) are also used.

4.5.8 Polysaccharides of microorganisms and higher fungi

Microorganisms and higher fungi produce two basic types of polysaccharides:

- **extracellular**
- **intracellular** (structural and storage polysaccharides).

Extracellular polysaccharides of bacteria are accumulated in the form of capsules, which remain part of the cell wall or as an amorphous mucilaginous material surrounding the outer cell wall, and diffuse into the growth medium. These **mucilages**, also known as **bacterial gums**, apper to have a barrier function to protect cells from infection by bacterial viruses (bacteriophages), prevent dehydration and fix microorganisms to the environment (e.g. to soil particles). Bacterial gums have unique properties for which they find use in the food industry, pharmaceutical industry and elsewhere. The most important bacterial extracellular hydrocolloid used for food purposes is **xanthan** (**xanthan gum**), while on a smaller scale **gellan** (gellan gum) is used. In addition to xanthan and gellan, a number of other bacterial polysaccharides are obtained, but for various reasons (e.g. low production, high cost and better resources) they are use only sporadically. Dextran and curdlan, for example, have limited uses. Bacteria of the genus *Azotobacter* are a potential source of bacterial alginates.

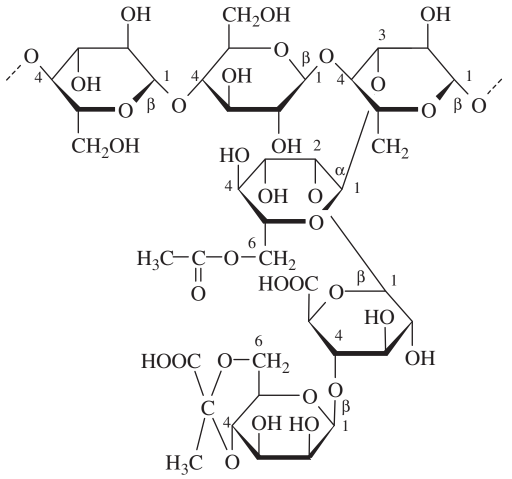
Yeasts, moulds and higher fungi produce a large variety of extra- and intracellular polysaccharides. Certain applications have been found for glucans as hydrocolloids in the food industry, especially α-glucans with multiple α-(1→4), α-(1→3) bonds and also some β-glucans with combined β-(1→3) and β-(1→6) bonds. Extracellular (excreted as mucilages) and intracellular (structural polysaccharides of cell walls) polysaccharides, which are commonly called **β-glucans**, or more precisely **β-(1→3)-D-glucans**, have unique properties. They are produced by some yeasts, moulds and higher fungi. Their antibacterial, antiviral, anticoagulant and

anticarcinogenic effects are mainly exploited by the pharmaceutical industry and in human medicine.

4.5.8.1 Xanthan

The extracellular polysaccharide xanthan (xanthan gum) is produced by bacteria of the genus *Xanthomonas* (industrially those most commonly used are the bacteria *X. campestris*).

The main xanthan chain consists of β-D-(1→4) glucose units that also occur in cellulose. Side chains (usually trisaccharides) consist of a D-glucuronic acid residue and two D-mannose residues (**4-180**). To the terminal D-mannose unit of the side chain is attached to β-D-glucuronic acid through β-(1→4) glycosidic bond. Glucuronic acid is further linked to α-D-mannose by a (1→2) bond. The structure is further complicated by the presence of pyruvic acid linked, as an



···· →4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→····
3
↑
1
β-D-Man*p*-(1→4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*6Ac
4 6
HOOC CH$_3$

4-180, basic structure of xanthan

acetal, at positions C-4 and C-6 of the terminal β-D-mannose unit, which is 4,6-*O*-(1′-carboxyethylidene)-β-D-mannopyranose. The internal mannose unit in the side chain is acetylated in position C-6 (6-*O*-acetyl-α-D-mannopyranose). The structure may also vary in the degree of substitution depending on the bacterial strain used in the production. The relative molecular weight is about 15 000 kDa. Xanthan molecules form single or double helices stabilised by side chains.

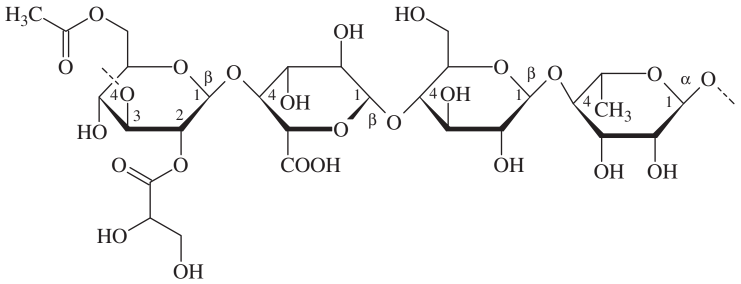
Xanthan gum is water soluble; its dispersions are highly viscous and even at low concentrations show thixotropic behaviour. The viscosity is strongly dependent on temperature. During heating, viscosity decreases at first, but then increases again with changes in the conformation of the molecules. Xanthan dispersions are stable in acidic and alkaline conditions and at elevated temperature (80 °C). In the presence of guar gum, the viscosity of dispersions increases, which is utilised in products that require a stable viscosity over a wide range of salt concentrations, pH and temperatures.

Xanthan does not form gels, but thermoreversible gels are formed in mixtures with some polysaccharides, such as galactomannans (locust gum), glucomannans (konjak gum) and κ-carrageenan. Gel formation requires the interaction of xanthan molecules (arranged in a double helix) with the unbranched part of another polysaccharide molecule (with its binding zone). Better, elastic, cohesive gels are formed from deacetylated xanthan.

Xanthan gum is used primarily as a thickener, emulsion stabiliser and in combination with other hydrocolloids as a gelling agent. The thermostability of xanthan is typically exploited in the preparation of instant soups, sauces and as binders in various canned foods.

4.5.8.2 Gellan

Gellan (gellan gum) is an extracellular polysaccharide produced commercially by aerobic submerged fermentation from bacteria *Sphingomonas elodea* (previously called *Pseudomonas elodea*). The gellan chain contains tetrasaccharide repeating structures β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→3) consisting of β-D-glucose esterified at position C-6 by acetic acid (the degree of substitution is about 50%) to which is bound L-glyceric acid at C-2 and D-glucuronic acid. D-Glucuronic acid is bound to the second molecule of D-glucose by a β-(1→4) bond, which is connected with α-L-rhamnose by a (1→4) bond (**4-181**).



···· →3)-β-D-Glc*p*6Ac-(1→ 4)-β-D-Glc*p*A-(1→ 4)-β-D-Glc*p*-(1→ 4)-α-L-Rha*p*-(1→ ····
2
↑
L-glyceric acid

4-181, basic structure of gellan

Gellan can occur also in a branched form, known as **welan**. The molecular weights range from thousands to tens of thousands of kilo daltons. Gellan gum forms coaxial triangular three-fold double helices from two left-handed chains coiled around each other, with the acetate residues on the periphery and glyceryl groups stabilising the interchain associations. The molecule is further stabilised by hydrogen bonds.

Gellan dissolves in water to form highly viscous solutions even at low concentration. The gelling properties depend on the degree of acetylation. The native acetylated product forms soft, elastic gels, whereas gellan produces hard and brittle gels. Gellan is an acidic polysaccharide and in the presence of cations, even at low (0.1% w/w) to very low (0.005% w/w) concentrations forms thermoreversible gels on cooling at about 50 °C. Gelatinisation depends on the cation type and its valence. Divalent cations form stronger and more elastic gels than monovalent cations.

Depending on their structure (degree of deacetylation), gellan gels form in either cold or warm water and are thermoreversible or thermoirreversible, which means they are perfect as broad spectrum gelatinisation agents that find use in many food and non-food applications (texture modification and stabilisation of foams and emulsions). They are used mainly in Japan. Gellan is used also in combination with other hydrocolloids, for example xanthan gum, locust gum and gelatine. In microbiology it is used as an alternative to agar gels due to its thermal stability (up to temperatures of 120 °C) and is particularly suitable for the cultivation of thermophilic microorganisms.

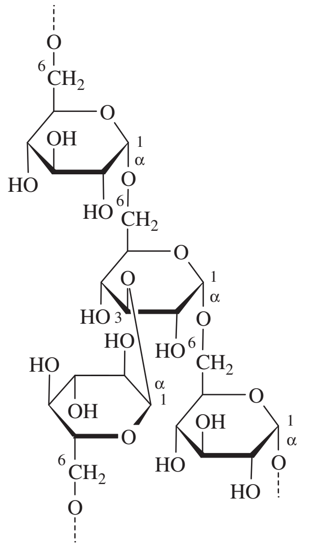
4.5.8.3 Dextran

The extracellular polysaccharide dextran is produced by the bacteria *Leuconostoc mesenteroides*, *Streptobacterium dextranicum*, *Streptococcus mutans* and some other bacteria. A dextran molecule consists of about 95% of α-(1→6) linked D-glucose units. The remainder are D-glucose molecules linked by α-(1→3) bonds that form side chains (**4-182**). Some dextrans contain side chains in which D-glucose molecules are also partly bound by α-(1→4) and α-(1→2) bonds. The type and number of these glycosidic bonds depends on the origin of the dextran. The relative molecular weight of dextran is usually 60–90 kDa.

Dextran is soluble in water, but water-insoluble dextrans also exist. Dextran dispersions have lower viscosity in comparison with xanthan dispersions, but are relatively resistant to hydrolysis.

In food applications, dextrans (*Leuconostoc mesenteroides* strains product) have been used, usually in combination with other polysaccharides such as gum arabic, for purposes similar to other hydrocolloids. They are highly effective emulsifiers and stabilisers of emulsions of oil in water. They bind water and inhibit the crystallisation of saccharose. Much more extensive use has been found for dextran in pharmacy and medicine (replacement of blood plasma) and in analytical chemistry as a chromatographic material for gel filtration and other applications.

Dextran naturally forms in contaminated sugar beet and juice, where higher viscosity can cause problems in sugar beet processing.



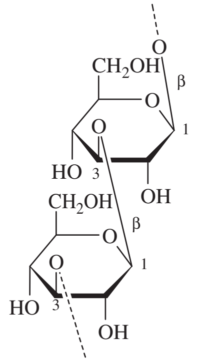
····→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→ ····
3
↑
1
···· →6)-α-D-Glc*p*

4-182, basic structure of dextran

4.5.8.4 Curdlan

Curdlan is an extracellular polysaccharide produced by the bacterium *Alcaligenes faecalis* var. *myxogenes*. It is also found in many yeasts and fungi as a component of cell walls or as a storage polysaccharide.

Curdlan is a linear polymer of glucose units linked by β-(1→3) bonds (**4-183**). The basic unit is actually disaccharide laminaribiose. It can be partially esterified by succinic acid. The relative molecular weight of curdlan is 44–77 kDa.



···· →3)-β-D-Glc*p*-(1 →3)-β-D-Glc*p*-(1→ ····

4-183, basic structure of curdlan

Curdlan swells in water and after heating to 80 °C forms a turbid gel that is stable over a wide range of temperatures and pH values. Gel strength can be influenced by the addition of sugars.

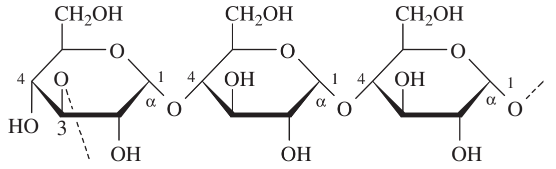
Curdlan serves as a gelling agent, thickener and stabiliser, it improves water binding capacity, viscoelasticity, masks various

odours and has an excellent ability to form films, which are insoluble in water, impermeable to oxygen and are biodegradable. It is mainly used in Japan.

4.5.8.5 Elsinan

Elsinan is an exocelullular glucan produced by the fungus *Elsinoe lencospila*, which forms white patches on the leaves of tea plants (*Camellia sinensis*, Theaceae), whose leaves and leaf buds are used to produce Chinese tea.

The elsinan chain is composed of D-glucose units linked by approximately 70% of α-(1→4) bonds and by about 28% of α-(1→3) bonds (**4-184**).



···· →3)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→ ····

4-184, basic structure of elsinan

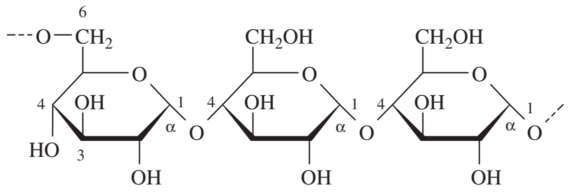
Elsinan is soluble in hot water. It is stable over a wide range of pH values and in the presence of salts, it can be hydrolysed by some amylolytic enzymes (e.g. by α-amylase), can form highly viscous solutions even at low concentrations and at concentrations higher than 5% it forms a gel. An important feature is the formation of strong, flexible films that are formed during evaporation of elsinan solutions.

Elsinan is used as low energy filler. Films made from elsinan are suitable as edible food packagings that are impermeable to oxygen.

4.5.8.6 Pullulan

Extracellular polysaccharide pullulan is produced by the fungus *Aureobasidium pullulans* (syn. *Pullularia pullulans*). The molecule consists of D-glucopyranose units linked alternately by two α-(1→4) and one α-(1→6) bonds (**4-185**). The molecular weight varies widely, from 1.5 to about 800 kDa, according to origin of the fungus and cultivation conditions.

Pullulan is soluble in water. The pressed polysaccharide has similar properties to polystyrene. When mixed with sorbitol or glycerol, pullulan forms translucent films that are impermeable to oxygen and fats.



···· →6)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→ ····

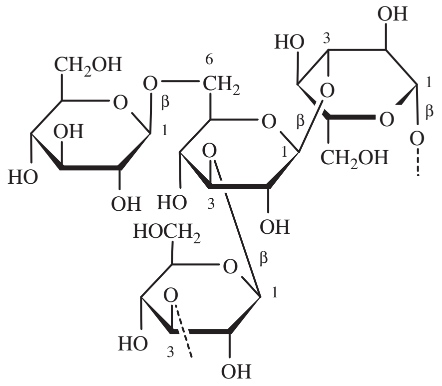
4-185, basic structure of pullulan

It is not used for food purposes. The most widespread applications of pullulan are in medicine (infusion solutions), pharmacy (coated tablets) and in the paper industry (adhesives).

4.5.8.7 Scleroglucan

Scleroglucan is the capsular polysaccharide excreted by some lower fungi of the genus *Sclerotium*. The fungi *S. glucanicum* and *S. roefsii* serve as an industrial source. Other sources are fungi of the genera *Sclerotinia*, *Corticium*, *Botrytis* and *Stromatinia* that produce polysaccharides similar to scleroglucan. Similar polysaccharides are also cell wall components and storage polysaccharides of yeasts and higher fungi.

Scleroglucan produced by *P. glucanicum* has a linear chain composed of D-glucose residues linked by β-(1→3) bonds, with side glucose residues linked with β-(1→6) bonds that occur on approximately every third glucose unit of the main chain (**4-186**). The polymers contain between one hundred and several hundred molecules of glucose.



···· →3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→ ····

6
↑
1

β-D-Glc*p*

4-186, basic structures of scleroglucan, schizophyllan and lentinan

Scleroglucan is soluble in water and forms highly viscous dispersions that are stable over a wide range of pH values and at higher temperatures. Dispersions of concentrations higher than 1.5% form gels. In combination with glycerol, scleroglucan forms a strong and flexible film and emulsions with vegetable oils.

The food use of scleroglucan is minimal; greater use is made in cosmetics, pharmaceuticals and in the manufacture of porcelain, paper and paints.

4.5.8.8 Other glucans

Polysaccharides structurally related to scleroglucan, which are β-glucans with glucose units in the main chain linked by (1→3) bonds and side chains attached by (1→6) bonds, are produced

as structural and storage (extra- and intracellular) polysaccharides by many yeasts, moulds and higher fungi. For example, yeast *Saccharomyces cerevisiae* contain as the major polysaccharides β-glucan with (1→6) bonds in the main chain and (1→3) bonds in the side chains and α-mannan with (1→6) bonds in the main chain and (1→2) and (1→3) bonds in the side chains. Cultivated yeast strains produce only β-glucan with (1→3) bonds in the main chain and (1→6) bonds in the side chains.

Polysaccharides composed of glucose units are also known as β-(1→3)-D-glucans. Individual polysaccharides differ in substitution of the main chain and the number of glucose units in the side chains. The chains form triple helices. β-(1→3)-D-Glucans are mostly isolated from so-called club fungi, belonging to the phylum Basidiomycota, and from so-called sac fungi of the phylum Ascomycota. Examples of these polysaccharides are given in Table 4.36.

The most important properties of β-(1→3)-D-glucans are their ability to strengthen the immune system and to inhibit tumour growth. Their activity depends on the molecular weight, frequency of branching and conformation of molecules. The highest efficiency reported is shown by β-D-glucans with a degree of branching of 0.20–0.33 and higher relative molecular weight (100–200 kDa). Some β-D-glucans (Table 4.36), for example schizophyllan (extracellular polysaccharide of Basidiomycota fungi) and lentinan (structural cell wall polysaccharide of Oomycota fungi) are used in clinical medicine as immunotherapeutic agents in the treatment of cancer diseases, often in combination with radiotherapy. Schizofyllan also protects against bacterial infections. Lentinan is used against viral diseases. The structure of both compounds is similar to the structure of scleroglucan (**4-186**) and differs only in the level of substitution of the main chain and the degree of polymerisation.

Schizofyllan is a product of phytopathogenic fungus *Schizophyllum commune* (Table 4.36), which produces three types of β-(1→3)-D-glucans with molecular weights 10, 20–80 and 200 kDa. The first two types contain disaccharide laminaribiose in the side chains. Glucans with a relative molecular weight of 200 kDa show the highest antitumor and cytotoxic activities. Their side chains contain trisaccharide β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*.

4.5.9 Animal polysaccharides

Among the polysaccharides of animal origin, the storage homopolysaccharide **glycogen** and structural homopolysaccharide **chitin** are important. In addition to these homopolysaccharides, animal tissues contain many other heteropolysaccharides, which have structural, protective and other functions.

4.5.9.1 Glycogen

The primary structure and function of glycogen resembles the starch component amylopectin and therefore glycogen is also called animal starch. Glycogen is the main utilisable source of glucose (energy) present in all animal cells, but also found in cells of bacteria, moulds and higher fungi.

4.5.9.1.1 Structure and nomenclature

Glycogen is an α-glucan that contains more than 10^6 D-glucose residues linked by α-(1→4) bonds. In every 8–12 glucose residues, side chains of other α-(1→4) glucans are bound, attached by α-(1→6) bonds, similar to as in amylopectin, but the glycogen molecule is more branched and structurally more compact. Glycogen contains also phosphoric acid in small amounts.

4.5.9.1.2 Occurrence

In mammals, glycogen is found mainly in the liver and muscles. The liver cells, hepatocytes, contain glycogen at a level of 2.9–8.1% of their weight (in total 100–120 g in adults). It occurs in the form

Table 4.36 Some β-D-(1→3)-glucans with important anticarcinogenic activity.

Name	Source: Latin name	Source: Other names	Degree of branching
AM-ASN	*Amanita muscaria*	Fly agaric (fly amanita) mushroom	0.3
β-Glucan I	*Auricularia auricula-judae*	Jew's ear (jelly ear) mushroom	0.75
Grifolan	*Grifola frondosa*	Hen of the woods (sheep's head) mushroom, Maitake mushroom (in Japan)	0.33
HA	*Pleurotus ostreatus*	Oyster mushroom	0.25
Lentinan	*Lentinula edodes*	Shiitake mushroom	0.23–0.33
Pachyman	*Wolfiporia extensa* (syn. *Poria cocos*)	Fu Ling mushroom (in China)	0.015–0.02
Schizophyllan	*Schizophyllum commune*	–	0.33
Scleroglucan	*Sclerotium glucanicum*	–	0.3
Tylopilan	*Tylopilus felleus*	Bitter bolete mushroom	0.33
Zymosan	*Saccharomyces cerevisiae*	Baker's yeast (brewer's yeast)	0.03–0.2

of granules in the cell cytoplasm. Approximately 1–2% of glycogen is also present in the skeletal muscles of animals (*in vivo*), but his total amount is higher than the content in the liver. Meat contains only about 0.15–0.18% of glycogen, with the exception of horse meat, which contains 0.9% glycogen.

Glycogen plays an important role in post-mortem changes in muscle. Anaerobic glycolysis transforms glycogen into L-lactic acid, which lowers the pH of the meat, increases its fragility during ripening and has antimicrobial effects, thereby extending the shelf life of meat during storage. The level of glycogen in the muscle of animals, poultry and fish prior to slaughter has a major impact on the quality of meat during further processing (see Section 2.4.5.1.5). In fish, glycogen is present at levels of up to 0.3%. It is also found in higher fungi, moulds, yeasts and bacteria. Higher fungi contain 5–10% glycogen. From a nutritional point of view, glycogen has little significance.

4.5.9.1.3 Properties

Glycogen is more soluble than starch and does not form gels.

4.5.9.1.4 Physiology and nutrition

The large number of non-reducing ends of the glycogen molecule play an important role in biochemical processes, as they allow rapid mobilisation of glucose during biodegradation of glycogen. In cells, glycogen is degraded for metabolic purposes by glycogen phosphorylase that splits α-(1→4) bonds from the non-reducing end of the molecule with the formation of glucose 1-phosphate. Glucose 1-phosphate is transformed into glucose 6-phosphate, which produces glucose via the process of glycolysis (with the catalytic action of hexokinase) or other sugar phosphates. The α-(1→6) bonds are cleaved by α-(1→6)-glucosidase (isomaltase).

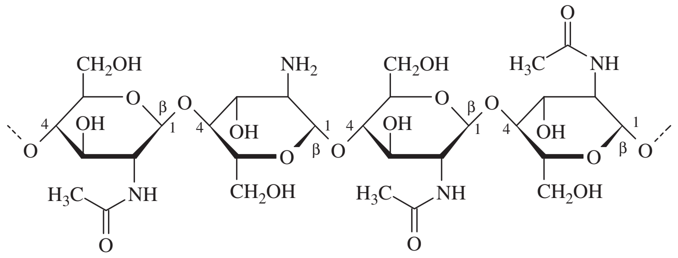
Glycogen is a utilisable polysaccharide. In food, it is hydrolysed by hydrolases (α-amylase and β-amylase) in the same way as starch. The branched dextrins formed are further broken down by isoamylase to maltose and glucose.

4.5.9.2 Chitin

4.5.9.2.1 Structure and nomenclature

Chitin is an almost linear copolymer of *N*-acetyl-β-D-glucosamine (70–90%) and β-D-glucosamine (10–30%), in which both monomers are bound to each other by β-(1→4) glycosidic bonds (**4-187**). The basic building block of chitin is disaccharide **chitobiose**, composed of two molecules of *N*-acetyl-β-D-glucosamine (also known as chitosamine). Its relative molecular weight is about 1000 kDa.

The chains are coiled along the longitudinal axis into a helix at certain distances (1–1.5 nm) and are oriented parallel or antiparallel. The most common conformation is an α-conformation in which the individual chains are arranged antiparallel. In the less frequent β-conformation, the chains are oriented in parallel.



····→4)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*N-(1→4)-β-D-Glc*p*NAc-(1→4)-β-D-Glc*p*NAc-(1→····

4-187, chitin

4.5.9.2.2 Occurrence

Chitin is the second most abundant organic compound in nature after cellulose. It is found mainly in the animal kingdom, where it is the main structural polysaccharide in the exoskeleton (shells) of crustaceans, insects and other invertebrates. It also occurs in some algae, fungi, yeasts and bacteria, usually associated with proteins. Naturally occurring chitin is consumed only rarely. Some beetles and sea foods with shells (such as crabs and snails) are eaten as delicacies, and are largely made of chitin. The chitin content in the exoskeleton of crabs is 61–77%.

The main sources of chitin in the diet are mainly the higher fungi that contain about 1% of chitin, such as cultivated common mushrooms (*Agaricus bisporus*) (1.3–8.0% of dry matter) and shiitake mushrooms (*Lentinula edodes*) containing 3.6–8.1% chitin in dry matter. Fermented soybeans and fermented rice used in the preparation of oriental foods contain chitin derived from moulds (*Aspergillus oryzae* and *A. sojae*). Fungi contain up to 42% chitin (e.g. *A. niger*). Baker's yeast (*Saccharomyces cerevisiae*) contains around 2.9% chitin, which becomes part of the baked products.

4.5.9.2.3 Properties

Chitin is insoluble in water and barely soluble in acidic media (forming ammonium salts). Heating in acid or alkaline solutions leads to the hydrolysis of bound *N*-acetyl-D-glucosamine with the formation of D-glucosamine and acetic acid. Heating under acidic conditions causes partial depolymerisation of chitin through the hydrolysis of β-glycosidic bonds. In alkaline solutions, depolymerisation occurs to a lesser extent.

The enzyme lysozyme cleaves β-(1→4) glycosidic bonds between *N*-acetylglucosamine and D-glucosamine. The more chitin is acetylated, the faster the reaction. The ability to decompose chitin is based on the antimicrobial effects of lysozyme. Some bacteria produce chitinases known as chitodextrinases or poly[β-1,4-(2-acetamido-2-deoxy-D-glucosidases)] and chitosanase (chitosan-*N*-acetylglucosamine hydrolases), which cleave chitin chains to form *N*-acetylglucosamine.

Chitin forms complexes with most transition metals. Copper ions form one of the strongest complexes. The rates of formation

and stability of the metal complexes depend on the temperature, the solubility of ions, their type, pH, degree of acetylation of chitin and other factors. Complexes with toxic metals (mercury or lead) can cause poisoning, because they break down in the digestive tract.

4.5.9.2.4 Physiology and nutrition

Chitin is virtually indigestible, because no human intestinal microflora contains the chitin cleaving enzymes. Chitin is only partially hydrolysed by saliva (by lysozyme) and in the stomach (by lysozyme and hydrochloric acid). A significant proportion of chitin can be utilised by some animals (e.g. by fish, fowl and rabbits).

4.5.9.2.5 Use

Chitin is obtained industrially mainly from the shells of marine molluscs. The technology involves the removal of accompanying proteins in dilute sodium hydroxide, calcium carbonate and hydrochloric acid. Partial alkaline hydrolysis of acetyl groups using sodium hydroxide is used for the preparation of modified chitin, which is called **chitosan**.

Chitosan

Chitosan contains 5–25% of *N*-acetylglucosamine and 75–95% of D-glucosamine units. Although the structures of chitin and chitosan differ only slightly, chemical reactivity and physical properties of these two polysaccharides are quite different. Chitosan is soluble in water, acids and organic solvents and insoluble in neutral and alkaline solutions (it is a stronger base than chitin). Chitosan molecules are polycations and coagulate when molecules or particles carrying negative charge (e.g. sodium alginate, sulfates, phosphates and proteins) are added to chitosan solutions. Dispersions of chitosan are highly viscous.

Chitosan is, like chitin, hydrolysed by acids, alkalis or enzymes and forms complexes with metals. Unlike chitin, chitosan reacts with aldehydes to form imines. It is also indigestible, but significantly reduces the cholesterol level in the blood serum and liver. The mechanism of this effect is not fully understood yet. Apparently, coagulation and flocculation of chitosan play an important role. Bile acids form micelles containing cholesterol, which are absorbed through the intestinal wall. Chitosan forms a gummy coagulate that captures and binds these micelles, which reduces the level of emulsified cholesterol. In the colon, chitosan blocks the conversion of cholesterol into coprostanol, which increases faecal excretion of cholesterol and reduces its transfer into the blood serum. Chitosan also forms salts with fatty acids, which do not dissociate even in acidic gastric juice. These salts bind lipids (triacylglycerols), which are thus excluded from the body.

Chitosan is used as an emulsifier (for margarine) and an emulsion stabiliser (it is added to hamburgers, ice creams and cheeses), thickener (viscosity increases), foam stabiliser, gelling agent, clarification agent for fruit juices and also in the pharmaceutical industry and in cosmetics.

4.6 Complex saccharides

In animal and plant tissues and in cells of microorganisms, saccharides are often part of more complex structures containing, simultaneously, proteins, peptides, lipids and other non-saccharide components. These saccharides are called **conjugated** or **complex** saccharides.

Peptides and proteins form a wide range of conjugated compounds with carbohydrates. Carbohydrates are present mostly as linear or branched oligosaccharides. In some conjugated compounds the properties of peptide (protein) predominate, therefore these complex saccharides include:

- **glycopeptides**
- **glycoproteins**.

In other complex carbohydrates the properties of saccharides predominate, and then these compounds are divided into:

- **peptidoglycans** (also known as **mucopeptides**)
- **proteoglycans** (also known as **mucoproteins**).

The linear polysaccharide components of peptidoglycans of bacterial cell walls, known as **mureins**, are made up of alternating units of *N*-acetyl-D-glucosamine and *N*-acetylmuramic acid that are linked by β-(1→4) glycosidic bonds. The residue of lactic acid in *N*-acetylmuramic acid is linked by an amide bond to L-alanine in tetrapeptide Ala-D-isoGlu-L-Lys-D-Ala. Saccharides in proteoglycans of higher animals are predominantly linear polymers, so-called **mucopolysaccharides**, composed of aldoses (D-galactose), glycuronic acids (D-glucuronic or L-iduronic acids), amino sugars (*N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine) and their sulfates.

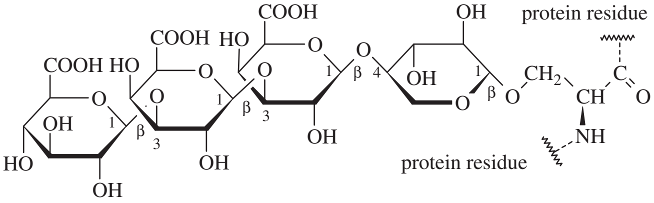
Saccharides also form a very diverse range of compounds with lipids. These compounds are categories of homolipids, heterolipids and complex lipids. Glycolipids are homolipids that include naturally occurring esters of higher fatty acids (e.g. glycoglycerolipids such as diacylgalactosylglycerol), synthetic sugar esters (e.g. esters of sucrose) and various sugar derivatives (such as glucitol esters). Heterolipids include some phospholipids (phosphatides, such as phosphatidylinositol) and lipamides (e.g. lipamide glycosides called cerebrosides). For example, complex lipids include mucolipids and gangliosides.

4.6.1 Mucopolysaccharides

Mucopolysaccharides, also known as **glycosaminoglycans**, are acidic linear polysaccharides composed of repeating disaccharide units. Mucopolysaccharides are sugar components of proteoglycans, peptidoglycans and also of glycoproteins and glycopeptides. Proteoglycans are essential components of the extracellular parts of the epithelial and connective tissues (skin, tendons, blood vessels, cartilages and bones) and also have protective functions in cells.

Glycoproteins are slimy and gelatinous substances in the synovial fluids of joints, liquids of eye sockets and in various secretions, such as respiratory tract mucus. They also occur in saliva, egg white and other viscous materials.

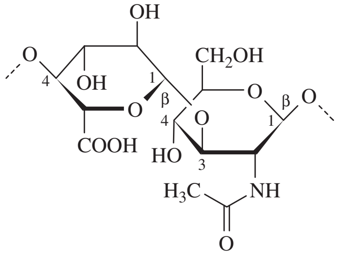
Glycosaminoglycans are covalently bound to proteins through a tetrasaccharide bound to the serine residue (**4-188**). Elongation of this tetrasaccharide (consecutive addition of sugar units to gluconic acid), esterification with sulfuric acid and various other reactions yield the individual glycosaminoglycans.



β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*-(1→4)-β-D-Xyl*p*-(1→

4-188, binding region of glycosaminoglycans

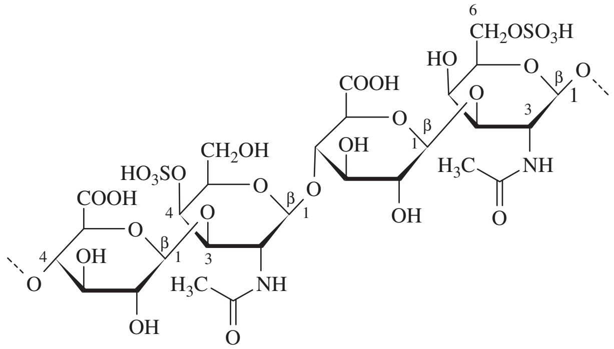
Hyaluronic acid is a common glycosaminoglycan that is not esterified with sulfuric acid. It consists of 250–25 000 alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine linked to each other by β-(1→3) glycosidic bond (**4-189**). Hyaluronic acid is present in the skin (which prevents penetration of bacteria into the tissues), in cartilage and the aqueous humour of the eyes resulting in the lubrication properties of synovial fluid. The properties are attributed to the formation of hydrogen bridges between the chains and their high degree of hydration. The enzyme hyaluronidase, hydrolysing β-(1→3) bonds, occurs in animal tissues, in bacteria (which is probably responsible for their invasiveness) and in snake and insect poisons.



… →4)-β-D-Glc*p*A-(1 →3)-β-D-Gal*p*N-(1→ …

4-189, basic structure of hyaluronic acid

There are two types of sulfated glycosaminoglycans. The first type includes galactosaminoglycans, known as chondroitin sulfate and dermatan sulfate, the second type are glucosaminoglycans, referred to as heparin sulfate and heparin. An example of a commonly occurring galactosaminoglycan is chondroitin sulfate, which contains alternating units of D-glucuronic acid and *N*-acetyl-D-galactosamine. The units of D-glucuronic acid can be esterified with sulfuric acid at position C-2, the units of *N*-acetyl-D-galactosamine are esterified at positions C-4, C-6 or they are

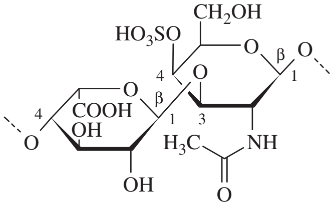


….→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc4SO_3-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc6SO_3^-(1 →..

4-190, basic structure of chondroitin sulfate

non-esterified (**4-190**). Chondroitin sulfate connects cells in animal tissues (mainly in epithelial and connective tissues).

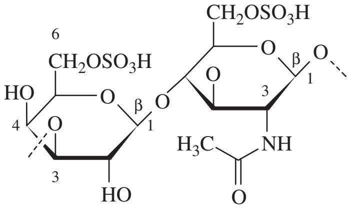
Dermatan sulfate differs from chondroitin sulfate as some units of D-glucuronic acid are replaced by L-iduronic acid units (**4-191**), or by its 2-sulfate and *N*-acetyl-D-galactosamine is esterified in position C-4 or C-6 (monoesters), simultaneously at C-4 and C-6 (diesters) or it is not esterified. Dermatan sulfate is found mostly in the skin (also in the blood vessels and tendons).



….→4)-β-L-Ido*p*A-(1→3)-β-D-Gal*p*NAc4SO_3^--(1→….

4-191, basic structure of dermatan sulfate

Instead of *N*-acetyl-D-galactosamine, keratan sulfate (kerato sulfate, **4-192**) contains *N*-acetyl-D-glucosamine and instead of D-glucuronic acid it contains D-galactose esterified at C-6 with sulfuric acid. *N*-Acetyl-D-glucosamine can also be esterified at C-6 with sulfuric acid. Other sugars are also present in small amounts, such as L-fucose, which is found in type II keratan sulfate. Keratan sulfate is part of cartilages and the cornea.



…. →3)-β-D-Gal*p*-(1 →4)-β-D-Glc*p*NAc6SO_3^--(1 → ….

4-192, basic structure of keratan sulfate

A related acidic heteropolysaccharide is heparin. It consists primarily of D-glucuronic acid (and also contains L-idurono-2-sulfate) and *N*-acetyl-D-glucosamine, which can be esterified with sulfuric acid at nitrogen, C-6, or both places simultaneously. These basic units are joined by α-(1→4) glycosidic bonds. Heparin does not occur in connective tissues, but is found in granular form in specific cells (particularly cells of the liver, lungs and skin) and is a part of the bloodstream. It has anticoagulant properties (prevents blood clotting). A very similar polysaccharide is heparan sulfate, which contains predominantly D-glucuronic acid (or L-iduronic acid and L-idurono-2-sulfate) and *N*-acetyl-D-glucosamine (or its sulfates as heparan). D-Glucuronic acid is linked by β-(1→4) glycosidic bonds with *N*-acetyl-D-glucosamine (its sulfates), which is linked with an additional unit of D-glucuronic acid by an α-(1→4) glycosidic bond. Disaccharide units are organised into sulfate and non-sulfate domains.

4.6.2 Proteoglycans

Mucopolysaccharides are covalently and non-covalently bound to various proteins to form macromolecules called proteoglycans or mucoproteins. The basis of the molecule of proteoglycans is a linear molecule of hyaluronic acid, to which are non-covalently attached proteoglycan subunits through the globular *N*-terminus. The subunits are composed of proteins covalently bound to mucopolysaccharides, mainly to chondroitin sulfate and keratan sulfate through serine and threonine hydroxyl groups or amide group of asparagine and the hemiacetal hydroxyl groups of *N*-acetyl-D-glucosamine or *N*-acetyl-D-galactosamine.

4.7 Reactions

4.7.1 Monosaccharides and oligosaccharides

The forms of sugars with free carbonyl groups and the cyclic forms are in equilibrium, and therefore most of the reactions of sugars involve the reactive carbonyl group and the anomeric hydroxyl group. Some reactions also involve primary and secondary hydroxyl groups of the chain (salt formation, formation of complexes with metals and other reactions). The presence of the hydroxyl groups allows carbohydrates to interact with the aqueous environment and to participate in non-bonding interactions.

Sugars in the enzymatically active food materials become often substrates for various enzymes that catalyse oxidoreduction reactions of sugars (oxidoreductases), their hydrolysis (hydrolases) and a range of other reactions.

The aldehyde group of aldoses is easily oxidised, yielding sugar acids (glyconic acids) that eliminate water and form lactones. Aldoses and ketoses isomerise under the catalysis of bases and break down to lower sugars and other compounds. Reduction of aldoses and ketoses yields sugar alcohols. In aqueous solutions (of pH 3–7) and in diluted solutions of acids, monosaccharides are relatively stable at normal temperatures. At higher temperatures, to a small extent, intermolecular condensation leads to disaccharides and higher oligosaccharides. The long-term effect of diluted acids or concentrated acids results in hydrolysis of polysaccharides and oligosaccharides, and monosaccharides undergo dehydration to furan and pyran derivatives. In alkaline solutions, monosaccharides are unstable and even at normal temperature they undergo isomerisation and elimination reactions and rearrangements, their carbon chain splits and recombines again. At higher temperatures, sugars decompose to low molecular weight products. Chemical reactions of monosaccharides are shown schematically in Figure 4.24.

Reactions of sugars in foods are generally complex biochemical (enzymatic) and chemical (non-enzymatic) reactions. All the functional groups of sugar molecules participate in these reactions, which are influenced by pH, temperature, water content and depend on many other factors, especially on the presence of other food components. In this respect, reactions of sugars themselves and reactions of sugars with other food components that result in the formation of brown pigments are generally classified as **non-enzymatic browning reactions**. The most important reaction is the reaction of reducing sugars with proteins, the **Maillard reaction**.

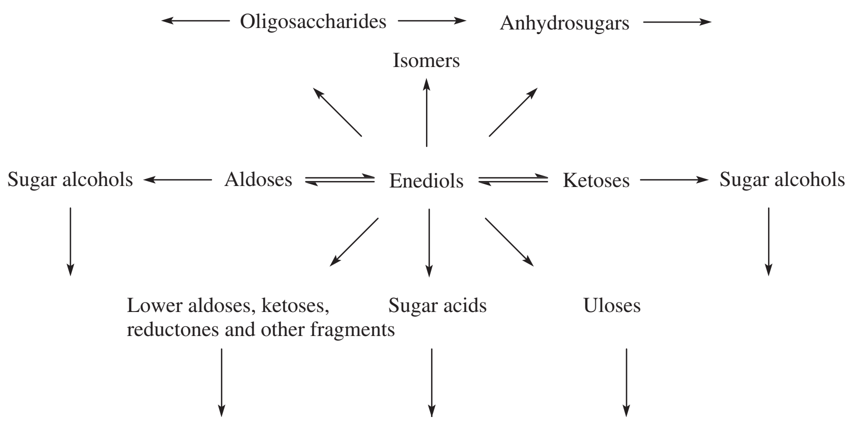


Figure 4.24 Main reactions of monosaccharides.

4.7.1.1 Reactions in acid media

4.7.1.1.1 Formation and hydrolysis of glycosides

Hemiacetal hydroxyl groups of pyranoses and furanoses are more acidic in comparison with other hydroxyl groups due to the negative inductive effect (–I effect) of the ring oxygen atom. Therefore, sugars tend to split these reactive hydroxyl groups in acidic media with the formation of mesomeric carbenium cations (Figure 4.25). This reaction is reversible. The existence of this cation explains a number of reactions that proceed on anomeric carbon, especially the formation and hydrolysis of glycosides.

A typical example is the formation of oligosaccharides from monosaccharides in acidic solutions. The reaction is called **reversion**. Its mechanism is opposite to the hydrolysis of oligosaccharides, which is **inversion**. The main products of D-glucose reversion are (1→6) disaccharides isomaltose and gentiobiose because the primary hydroxyl group on the C-6 carbon is more reactive than the secondary hydroxyl groups. Of the total products of glucose reversion, isomaltose amounts to about 68–70% and the gentiobiose to 17–18%. Minor reaction products are (1↔1), (1→2), (1→3) and (1→4) disaccharides and some higher oligosaccharides. Reversion products are commonly found in hydrolysates of saccharose, starch and other polysaccharides and arise also during caramelisation of sugars. For example, the reversion products in hydrolysates of starch are about 5–6%.

Glycosides have the typical properties of acetals. They are generally stable in alkaline solutions, but are hydrolysed in acidic solutions to the parent sugar and aglycone. Hydrolysis of glycosides by acids starts with protonation of the aglycone oxygen atom (Figure 4.25). Hydrolysed β-glycosides are generally hydrolysed faster than α-glycosides; the reaction rate depends considerably on the sugar moiety substituents. Hydrolysis of glycosides containing a 2-deoxysugar with hydrogen as a C-2 substituent is relatively rapid. For sugars containing either the electronegative hydroxyl group (which the majority of important sugars have) or the protonated amino group (in 2-amino-2-deoxysugars) in position C-2 hydrolysis is much slower.

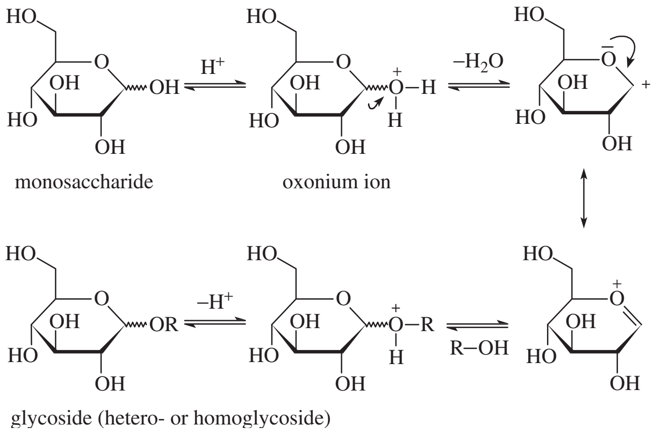


Figure 4.25 Mechanism of glycoside formation and hydrolysis.

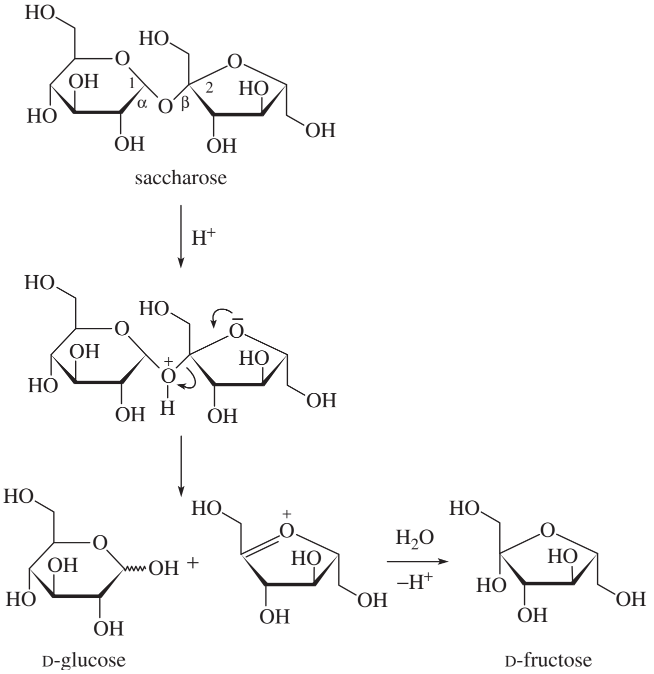


Figure 4.26 Mechanism of saccharose hydrolysis.

One of the most stable disaccharides is α,α-trehalose that contains two C-2 hydroxyl groups and is therefore about ten times more stable than maltose or lactose. Increased stability determines, in particular, the negative inductive effect of the hydroxyl groups at C-2. The hydrolysis rate of the galactose-*O*-glucose bond in raffinose, and also of the glucose-*O*-fructose bond in the reducing disaccharide turanose (Table 4.14), is comparable to the hydrolysis rate of maltose or lactose.

Hydrolysis (inversion) of saccharose to glucose and fructose is about 10^3 times faster than hydrolysis of other disaccharides, such as maltose and lactose (the rate constant of this first-order reaction in solutions of pH 1–3 at 100 °C is approximately $1\times10^{-3}\ s^{-1}$). Hydrolysis of saccharose takes place even at low temperatures, in the presence of diluted mineral or organic acids and in the presence of even small amounts of water. The glycosidic bond in glucose-*O*-fructose in raffinose, which yields melibiose (Table 4.16), is hydrolysed at a similar rate as saccharose. The reason is that the mechanism of hydrolysis of saccharose is somewhat different from the mechanism of hydrolysis of other glycosidic bonds (such as glucose-*O*-glucose or galactose-*O*-glucose). It is assumed that the resulting cation is degraded to glucose and a stable five membered cation of fructose. The reaction of this cation with water forms fructose (Figure 4.26).

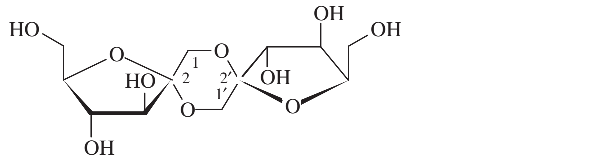
4.7.1.1.2 Formation of anhydrosugars

In addition to extramolecular condensation to homoglycosides, aldohexoses also undergo intramolecular condensation of the hemiacetal and other hydroxyl groups to form the corresponding anhydrosugar (sugar anhydride). In addition to the main

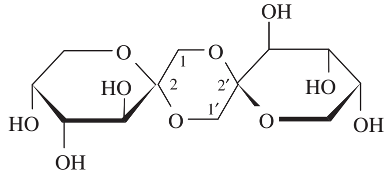
reaction product, 1,6-anhydropyranose, small amounts of 1,6-anhydrofuranoses and other anhydrosugars may also form.

In acidic solutions D-glucose yields about 1% of 1,6-anhydro-β-D-glucopyranose known as β-glucosan or laevoglucosan. It is also produced under partial polymerisation to branched polysaccharides when glucose syrup is heated to temperatures higher than 100 °C, and by pyrolysis of glucose, starch and cellulose. Acids hydrolyse β-glucosan to D-glucose. The mechanism of formation of anhydrosugars from D-glucose is shown in Figure 4.27. Under similar conditions D-mannose yields 1,6-anhydro-β-D-mannopyranose (β-mannosan) and D-galactose yields 1,6-anhydro-β-D-galactopyranose (β-galactosan). The pyranose ring of 1,6-anhydrosugars is fixed in the $^1C_4$ conformation. Anhydrosugars of those monosaccharides that have a higher number of equatorial hydroxyl groups with appropriate conformation, such as β-D-idopyranose, are formed in larger amounts.

Ketoses do not form monomeric anhydrides, but intermolecular reactions yield tricyclic compounds. Heating of D-fructose (and also heating of saccharose or inulin) gives rise to various dianhydrides of the corresponding furanoses and pyranoses. Examples of these intermolecular anhydrides formed from fructose are the 1,2′:1′,2-dianhydride of α-D-fructofuranose with α-D-fructofuranose (**4-193**) and 1,2′:1′,2-dianhydride of α-D-fructopyranose with β-D-fructopyranose (**4-194**). Anhydrides are also formed by dehydration of disaccharides and sugar alcohols.



4-193, (α-D-fructofuranose)-(α-D-fructofuranose)-1,2′:1′,2-dianhydride



4-194, (α-D-fructopyranose)-(β-D-fructopyranose)-1,2′:1′,2-dianhydride

4.7.1.1.3 Formation of furan and pyran derivatives

The main reaction of monosaccharides under strongly acidic conditions is dehydration, associated with the loss of 1–3 molecules of water and the formation of furan and pyran derivatives. Both types of compounds are also formed during caramelisation of sugars. In practice, dehydration of monosaccharides and formation of furan and pyran derivatives also occurs in slightly acidic solutions, such as during long-term storage of fruit compotes, especially at elevated temperatures. Higher levels of furans and pyrans are formed during hydrolysis of starch, and significant amounts of these compounds arises in the production of acid protein hydrolysates that are used as seasonings for soups, sauces, gravies, snacks, meat and other products.

The reaction mechanism of the formation of furans and pyrans from D-glucose and D-fructose (ketoses are more reactive than aldoses) is shown in Figure 4.28. The intermediate product of dehydration of both sugars is 1-ene-1,2-diol (1-ene-1,2-diolate) stabilised by hydrogen bonds (Figure 4.29). The formation of 1-ene-1,2-diolate is known as **1,2-enolisation**. The reaction involves acid/base catalysis, so endiolates are common products of acid catalysed dehydration as well as isomerisation, saccharinic acid re-arrangement and fragmentation of sugars in alkaline media. All these reactions are often collectively called Lobry de Bruyn–Alberda van Ekenstein transformations of aldoses and ketoses.

The elimination of one molecule of water from D-glucose or D-fructose produces 3-deoxy-D-2-glycos-2-ulose, also known under the systematic name 3-deoxy-D-*erythro*-hexos-2-ulose (or

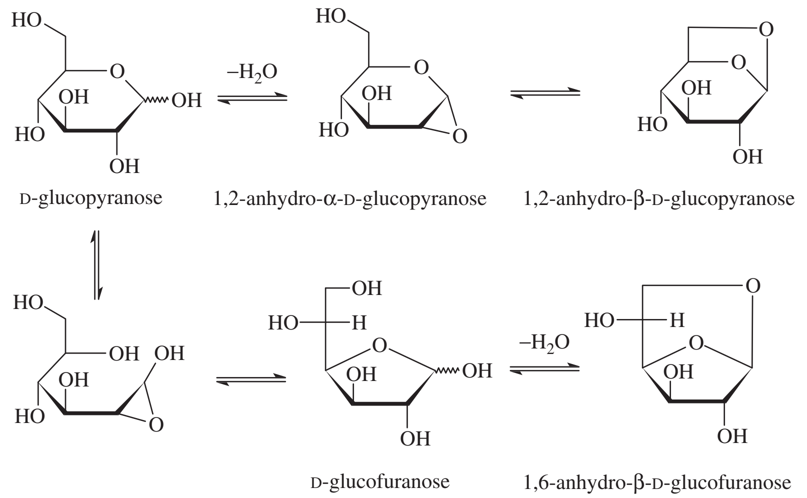


Figure 4.27 Formation of glucose anhydrides.

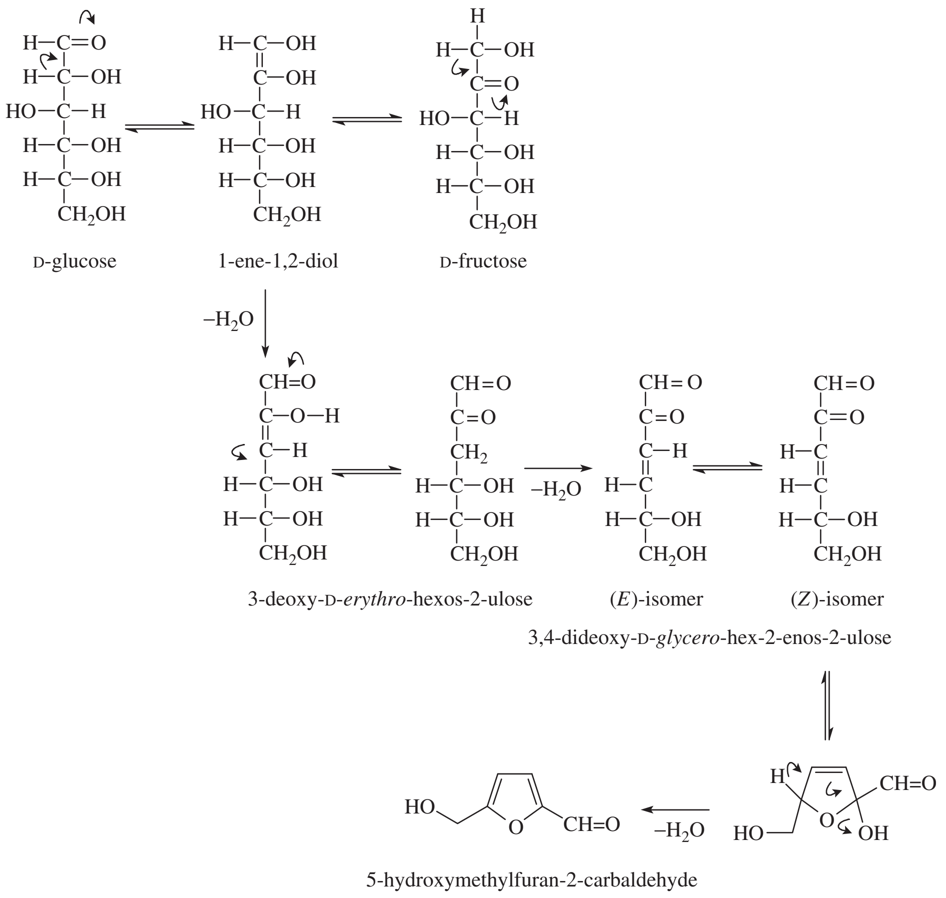


Figure 4.28 Dehydration of glucose and fructose.

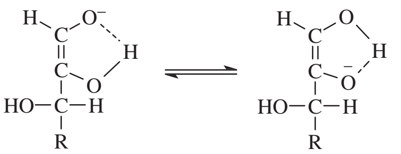


Figure 4.29 Structure of (*Z*)-1-ene-1,2-diolate.

by older name 3-deoxy-D-glucosone). In analogy, 3-deoxy-D-*threo*-hexos-2-ulose (3-deoxy-D-galactosone) results from D-galactose in milk products. Loss of the second water molecule creates 3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose (3,4-dideoxy-D-glucosulos-3-ene). The elimination of the third water molecule then yields 5-hydroxymethylfuran-2-carbaldehyde that can further decompose to laevulinic and formic acids. 5-Hydroxymethylfuran-2-carbaldehyde is one of the most common products of the dehydration of hexoses. It is virtually absent in fresh and untreated foods, but occurs in many carbohydrate-rich processed foods. For example, the concentrations of 5-hydroxymethylfuran-2-carbaldehyde in honeys were 3.3–26.3 mg/kg, in apple juices 2.9–3.5 mg/kg, in orange juices 2.7–10.6, in jams 2.7–15.9 mg/kg, in orange marmalade 47.1 mg/kg, in toasts 41.7–591 mg/kg and in roasted coffee 113–1093 mg/kg. When heated with hydrochloric acid, as in the production of acid hydrolysates of proteins (hydrolysis is performed at a temperature of 100–120 °C in about 20% acid), hexoses yield 5-chloromethylfuran-2-carbaldehyde (about 1–2 mg/kg) as a minor product.

The formation of 5-hydroxymethylfuran-2-carbaldehyde from saccharose proceeds via hydrolysis to fructofuranosyl cation and glucose (Figure 4.26). Elimination of a proton and two molecules of water from the fructofuranosyl cation yields 5-hydroxymethylfuran-2-carbaldehyde. Under dry pyrolytic conditions and at temperatures above 250 °C, 90% of 5-hydroxymethylfuran-2-carbaldehyde originates from the fructose moiety and only 10% originates from glucose. Pentoses and L-ascorbic acid dehydrate in the same way as hexoses under acidic conditions yielding furan-2-carbaldehyde (via reactive 3-deoxy-L-*threo*-pentos-2-ulose and 3,4-dideoxypentosulos-3-ene) as the main product (see Section 5.14.6.1.5). 6-Deoxyhexoses, such as L-rhamnose, yield, analogously, 5-methylfuran-2-carbaldehyde (**4-195**).

3-Deoxy-D-*erythro*-hexos-2-ulose occurs mainly as α- or β-pyranose in aqueous solutions. Owing to the presence of a keto group at C-2, other cyclic forms may also be present, such as furanoses (shown in Figure 4.30). In weakly acidic and neutral solutions, 3-deoxy-D-*erythro*-hexos-2-ulose eliminates water and yields γ-lactone of metasaccharinic acid and other products

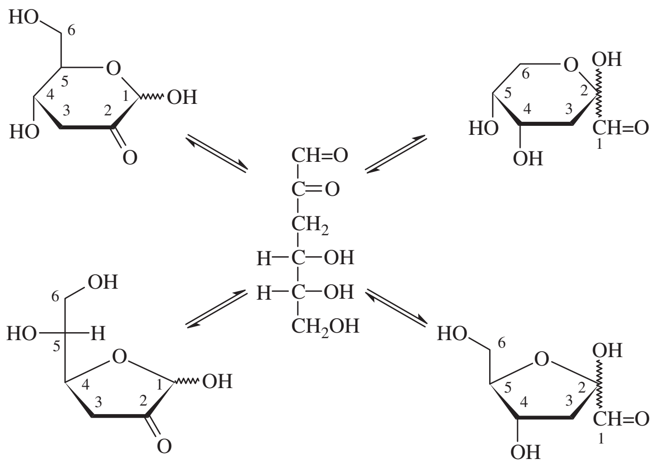
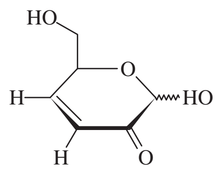
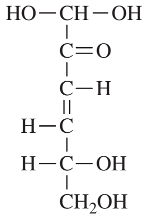


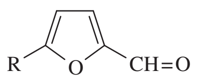
Figure 4.30 Cyclic forms of 3-deoxy-D-*erythro*-hexos-2-ulose in solutions.



4-196, (*Z*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose



4-197, (*E*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose hydrate



4-195, furan-2-carbaldehydes

furan-2-carbaldehyde, R = H
5-methylfuran-2-carbaldehyde, R = CH_3
5-chloromethylfuran-2-carbaldehyde, R = CH_2Cl

(Figure 4.31). Metasaccharinic and other sugar acids are, however, formed, preferably in alkaline solutions. Pyranoses also dominate in (*Z*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose (**4-196**) solutions. The existence of a six-membered ring in (*E*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose is not possible; therefore in aqueous solutions this isomer occurs as a straight chain form or as its hydrate (**4-197**), respectively.

5-Hydroxymethylfuran-2-carbaldehyde is the main reaction product of glucose and fructose in strongly acidic solutions, but fructose degradation also produces some other furan derivatives. Enolisation of D-fructose may continue along the chain and isomerisation via 2-ene-2,3-diol leads to a wider range of products than in the case of glucose. This isomerisation is known as **2,3-enolisation**. Splitting of the hydroxyl group at C-4 probably yields, by a sequence of isomerisation and dehydration reactions via 4-deoxy-D-*glycero*-hexo-2,3-diulose, another important degradation product of fructose 2-hydroxyacetylfuran, also known as furyl hydroxymethyl ketone (Figure 4.32). Via 1-deoxy-D-*erythro*-hexo-2,4-diulose (an isomerisation product of 1-deoxy-D-*erythro*-hexo-2,3-diulose, Figure 4.36) and 1,4-dideoxy-D-*glycero*-hexo-2,3-diulose (a product of oligosaccharides degradation, see also Figure 4.95) another dehydration product of sugars 2-acetylfuran is formed. The reactivity of sugars in 2-acetylfuran formation decreases

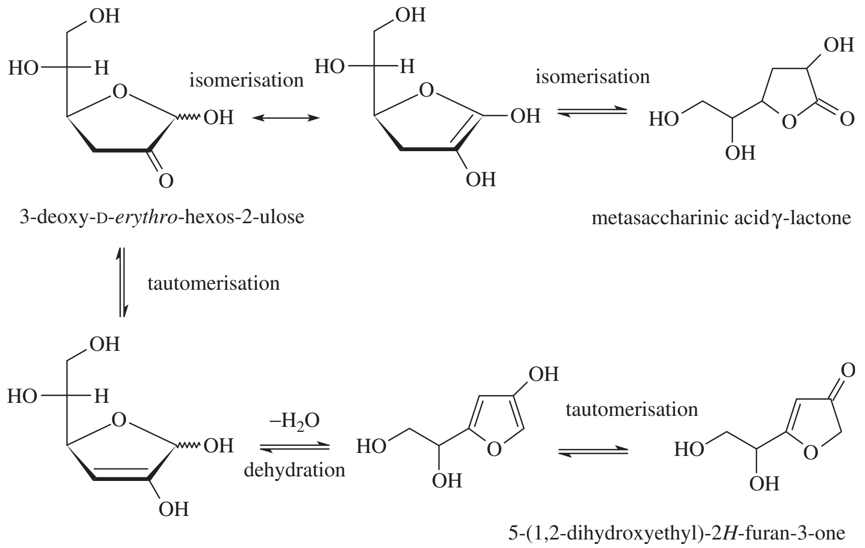


Figure 4.31 Formation of metasaccharinic acids and furan-3-ones from 3-deoxy-D-*erythro*-hexos-2-ulose.

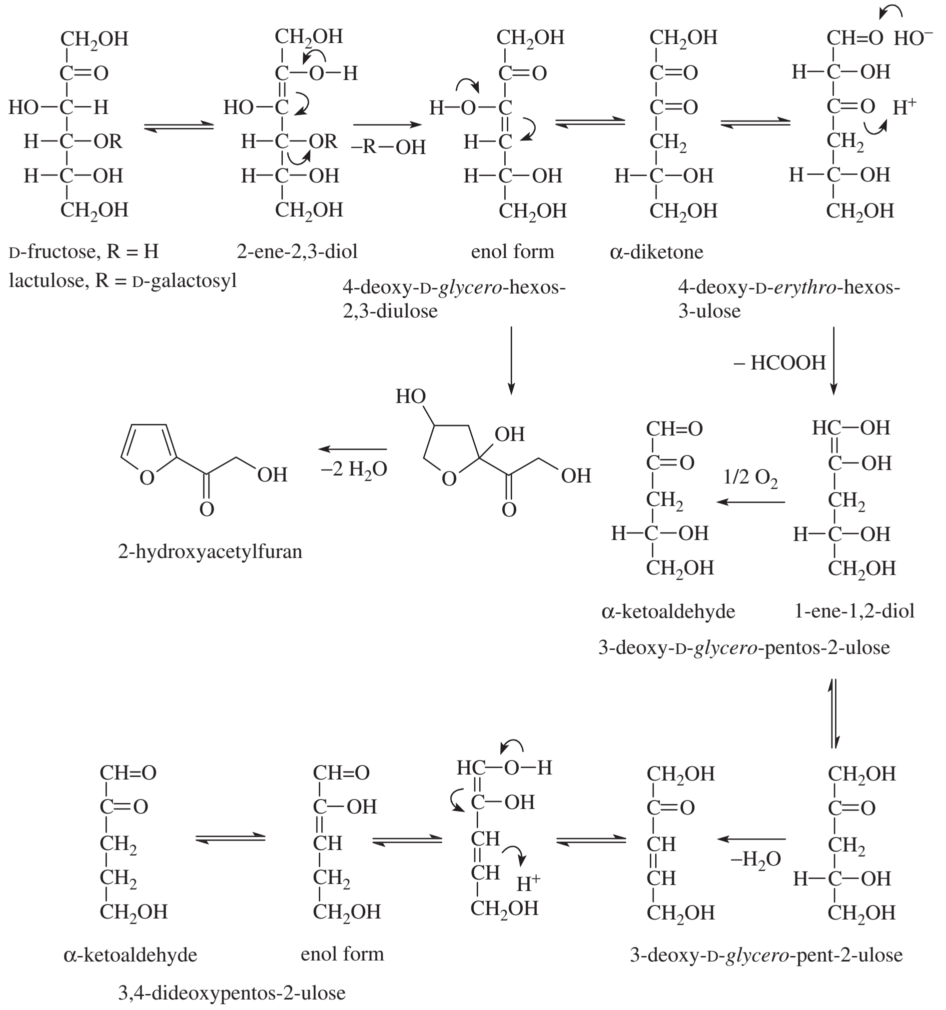


Figure 4.32 Formation of 2-hydroxyacetylfuran and 3-deoxy-D-*glycero*-pent-2-ulose from fructose and lactulose.

in the order ribose, fructose, glucose, rhamnose and sucrose. 1-Deoxy-2,3-diulose derived from ribose (and ascorbic and dehydroascorbic acids) reacts with formaldehyde (formed, for example, in the Strecker degradation of glycine) before forming a six-carbon unit – 1-deoxy-D-*erythro*-hexo-2,4-diulose – leading to 2-acetylfuran (Figure 4.33).

4-Deoxy-D-*glycero*-hexo-2,3-diulose is a typical product of degradation and 4-*O*-substituted derivatives of fructose (e.g. lactulose, which arises by isomerisation of lactose). 1,2-Enolisation of 4-deoxy-D-*glycero*-hexo-2,3-diulose yields, via the appropriate 1-ene-1,2-diol, unstable 4-deoxyhexos-3-ulose, which splits into formic acid and 1,2-enediol of 3-deoxy-D-*glycero*-pentulose in neutral or alkaline solutions by a reverse Claisen condensation mechanism. This sugar can be oxidised to 3-deoxy-D-*glycero*-pentos-2-ulose or may dehydrate to form 3,4-dideoxypentosulose (Figure 4.32). Other reaction products are isosaccharinic acids.

Splitting of the hydroxyl group at C-1 of fructose yields a key intermediate 1-deoxy-D-*erythro*-hexo-2,3-diulose (Figure 4.34). In addition to the straight chain form, the corresponding furanoses and pyranoses also form in solution (Figure 4.35). Isomerisation of 1-deoxy-D-*erythro*-hexo-2,3-diulose along the chain yields other important intermediates: 1-deoxy-D-*erythro*-hexo-2,4-diulose, 1-deoxy-D-*erythro*-hexo-3,4-diulose, 1-deoxy-D-*erythro*-hexo-3,5-diulose, 1-deoxy-D-*erythro*-hexo-4,5-diulose and 1-deoxy-D-*ribo*-hexo-4,6-diulose (Figure 4.36). 1-Deoxy-D-*erythro*-hexo-2,3-diulose is considered a precursor of many important furanones and pyranones that arise particularly in weakly acidic and neutral solutions. For example, this diulose is the precursor of 3-hydroxy-2-methylpyran-4-one, which is trivially termed maltol, and of its isomer 2-acetyl-3-hydroxyfuran, known as isomaltol (Figure 4.37). Maltol and isomaltol have the characteristic and intense caramel odour and taste.

In comparison with hexoses, higher quantities of maltol and isomaltol are produced from 4-*O*-substituted glucose derivatives, such as from maltose and lactose. For example, in malt coffee (coffee substitutes), maltol occurs at a level of 300 mg/kg, in chocolates at approximately 3 mg/kg and in dark beers up to 3 mg/kg. The mechanism of degradation of 1-deoxyglycodiuloses derived from disaccharides is somewhat different from that of 1-deoxyglycodiuloses arising from monosaccharides (Figure 4.38). Elimination of the hydroxyl group in position C-5 of the cyclic diulose (pyranose) yields pyran-3-one. This compound,

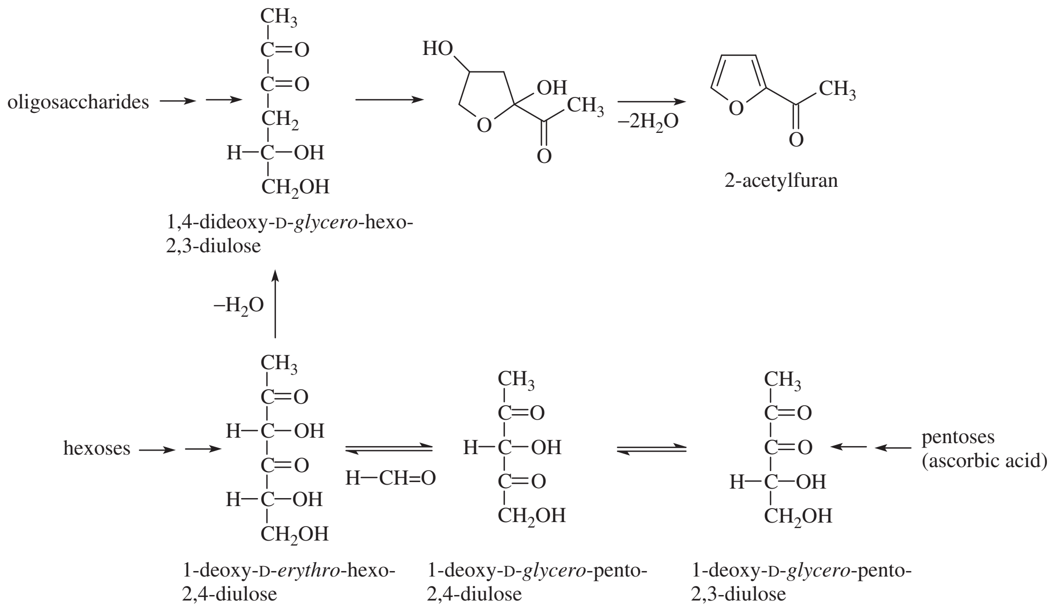


Figure 4.33 Formation of 2-acetylfuran from pentoses, hexoses and oligosaccharides.

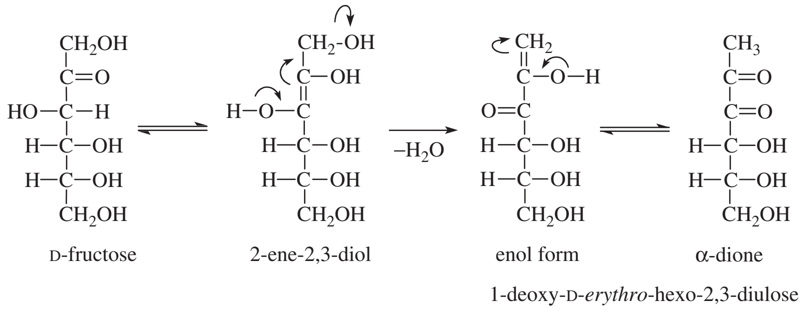


Figure 4.34 Formation of 1-deoxy-D-*erythro*-hexo-2,3-diulose from fructose.

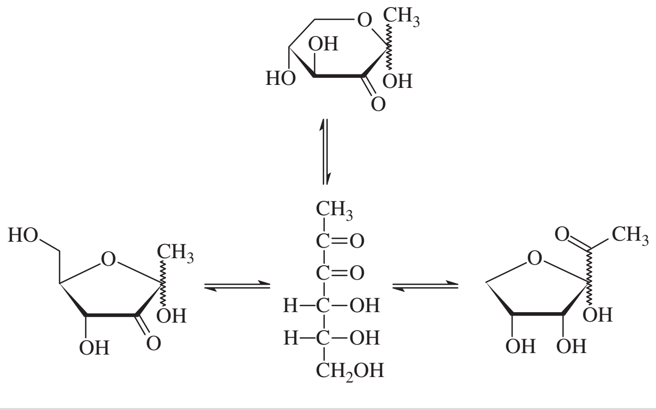


Figure 4.35 Cyclic forms of 1-deoxy-D-*erythro*-hexo-2,3-diulose.

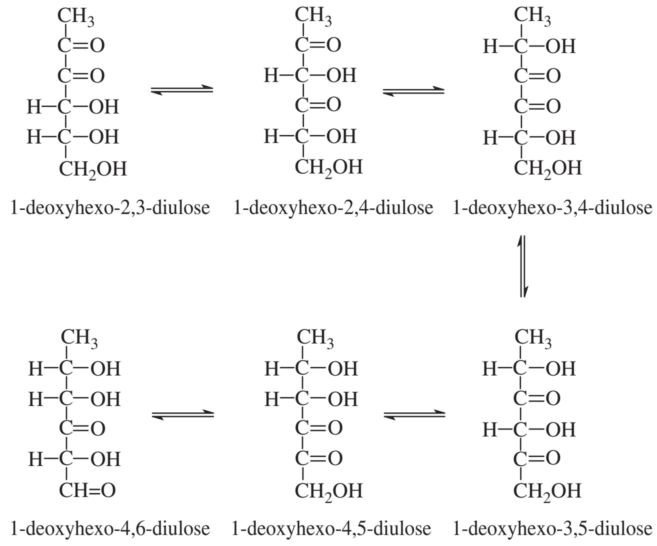


Figure 4.36 Products of 1-deoxy-D-*erythro*-hexo-2,3-diulose isomerisation.

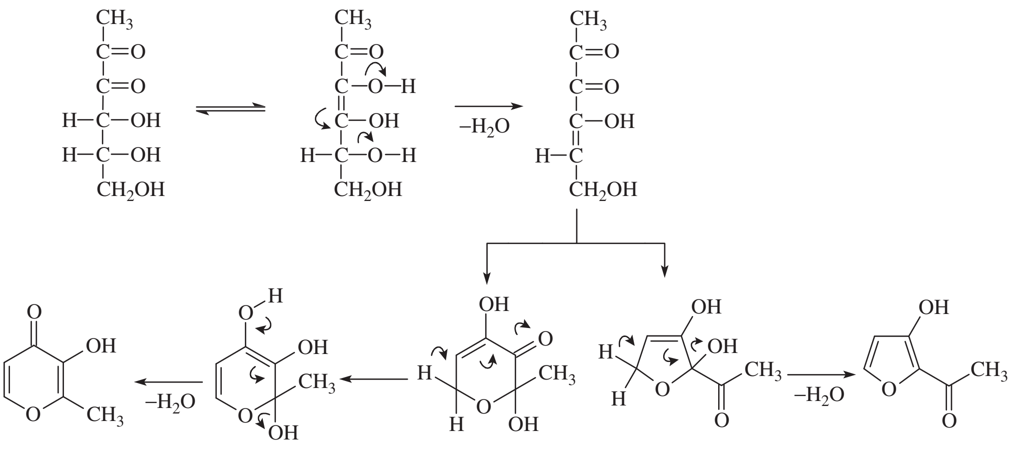


Figure 4.37 Formation of maltol and isomaltol from monosaccharides.

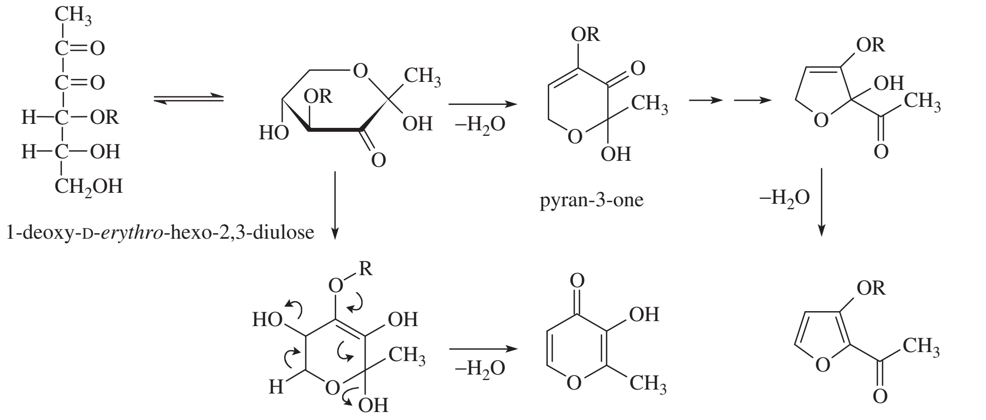


Figure 4.38 Formation of maltol and isomaltol from disaccharides.

which was also found in heat-treated milk (R = β-D-galactosyl), is the key intermediate of the degradation of disaccharides. The fate of this intermediate depends on the properties of glycosidically bound sugar. If that sugar is β-galactose (in the case of lactose), the main reaction product is galactosylisomaltol. If that sugar is glucose (in the case of maltose), maltol and glucosylisomaltol are formed only in trace amounts. For example, glucosylisomaltol was determined in prebaked bread (heated at 190 °C for 30 min), where its amount increased from non-detectable to 20.9 mg/kg after 30 min of baking.

A compound with similar organoleptic properties to those of isomaltol or maltol is 2,4-dihydroxy-2,5-dimethyl-2*H*-furan-3-one, the cyclic form of so called diacetylformoin. Diacetylformoin arises from 1-deoxy-D-*erythro*-hexo-2,3-diulose (Figure 4.39) via 1,6-dideoxy-D-*glycero*-hexo-2,4,5-triulose (acyclic form of diacetylformoin) and other intermediates (Figure 4.40). Diacetylformoin is considered a precursor of other important furans and pyrans and of many low molecular weight degradation products. In addition to diacetylformoin, hexoses (cyclic forms of 1-deoxy-D-*erythro*-hexo-2,3-diulose) also give rise to 4-hydroxy-5-hydroxymethyl-2-methyl-2*H*-furan-3-one and to isomeric 5,6-dihydro-3,5-dihydroxy-2-methyl-4*H*-pyran-4-one (Figure 4.39). 5,6-Dihydro-3,5-dihydroxy-2-methyl-4*H*-pyran-4-one is typically formed in thermally processed foods and its presence indicates the extent of non-enzymatic browning reactions. This dihydropyranone is related to maltol, but maltol is not produced from this compound under the conditions of the Maillard reaction. Along with 5,6-dihydro-3,5-dihydroxy-2-methyl-4*H*-pyran-4-one, its unstable isomer, the corresponding pyran-3-one is also formed, but this compound rearranges to lactic acid ester.

The reaction product of pentoses, hexoses (e.g. D-fructose via 1-deoxy-D-*erythro*-hexo-2,3-diulose) or alduronic acids is 4-hydroxy-5-methyl-2*H*-furan-3-one, which is known as norfuraneol (Figure 4.39). Norfuraneol can also be formed from hexoses via 4-hydroxy-2-hydroxymethyl-5-methyl-2*H*-furan-3-one by splitting off formaldehyde. 6-Deoxyhexoses (methylpentoses) produce, analogously, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one, which is known as furaneol (Figure 4.39). An alternative reaction

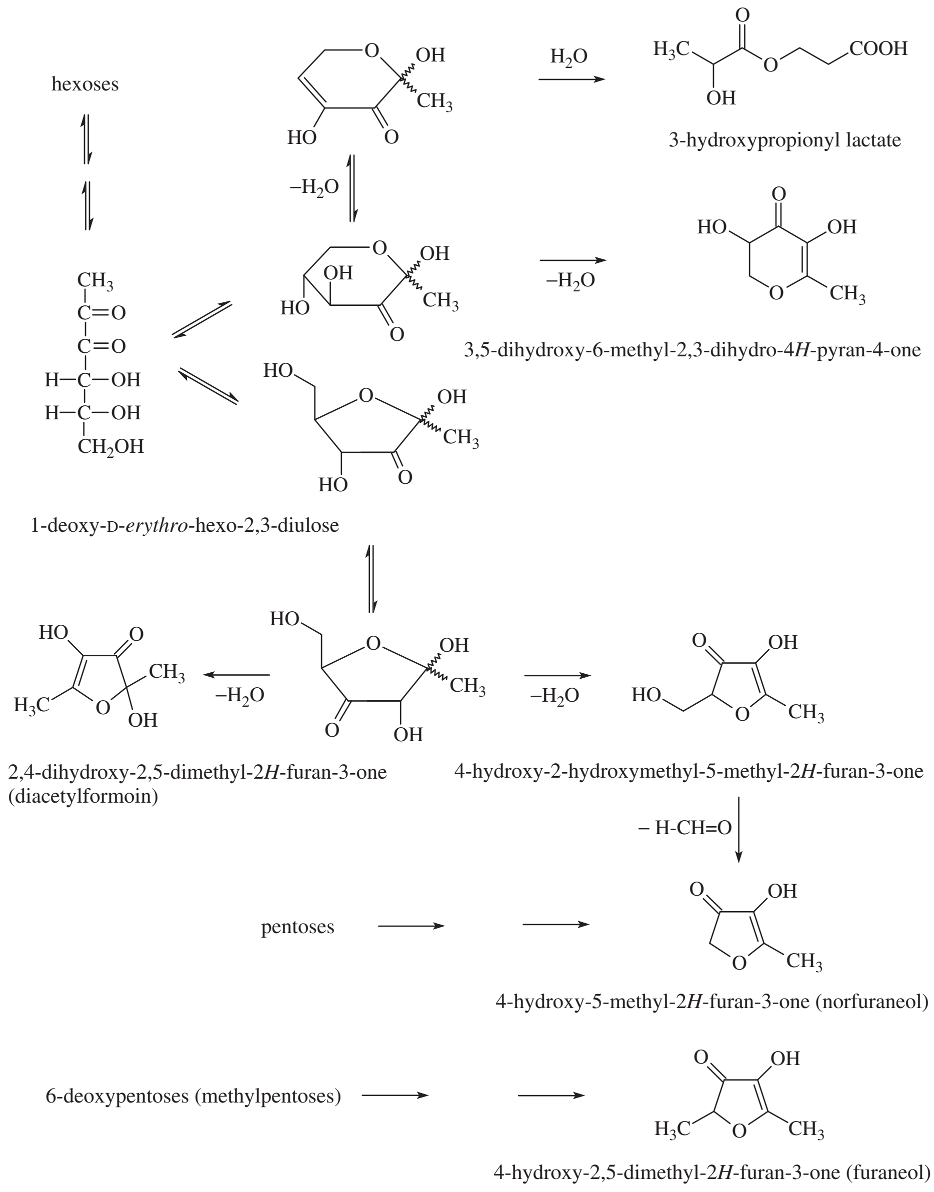


Figure 4.39 Formation of furan-3-ones and pyran-4-ones.

pathway to 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one is a reaction of methylglyoxal with hydroxyacetone (1-hydroxypropan-2-one) (Figure 4.41). Furaneol occurs as a racemic mixture because of its configurational instability (keto–enol tautomerism).

Furaneol and norfuraneol are significant sensorially active substances with low threshold concentrations. They are synthesised and added to numerous aroma compositions with a pleasant sugary, jammy, fruity and caramel flavour reminiscent of cooked strawberries and pineapple, which is where furaneol was identified for the first time (see Section 8.2.11.1.1).

4.7.1.1.4 Subsequent reactions of furans and pyrans

Under acidic conditions, 5-hydroxymethylfuran-2-carbaldehyde partially decomposes to laevulinic (4-oxopentanoic) acid and formic acid (Figure 4.42). Both acids are, for example, important components of acid protein hydrolysates. Laevulinic acid content in acid protein hydrolysates can be up to about 2% w/w. Its cyclisation product, α-angelica lactone, was identified in acid hydrolysates of proteins, extracts from vanilla pods, raisins, bread and other products.

All derivatives of furan-2-carbaldehyde are reactive substances. They are easily oxidised by oxygen to the corresponding acids, which, together with the appropriate alcohols, are also formed from furan-2-carbaldehyde derivatives by Cannizzaro reaction. Derivatives of furan-2-carbaldehyde condense together or with other carbonyl compounds and enter into non-enzymatic browning reactions. Oxidation of 5-hydroxymethylfuran-2-carbaldehyde, for example, yields 5-hydroxymethylfuran-2-carboxylic acid (**4-198**). Condensation

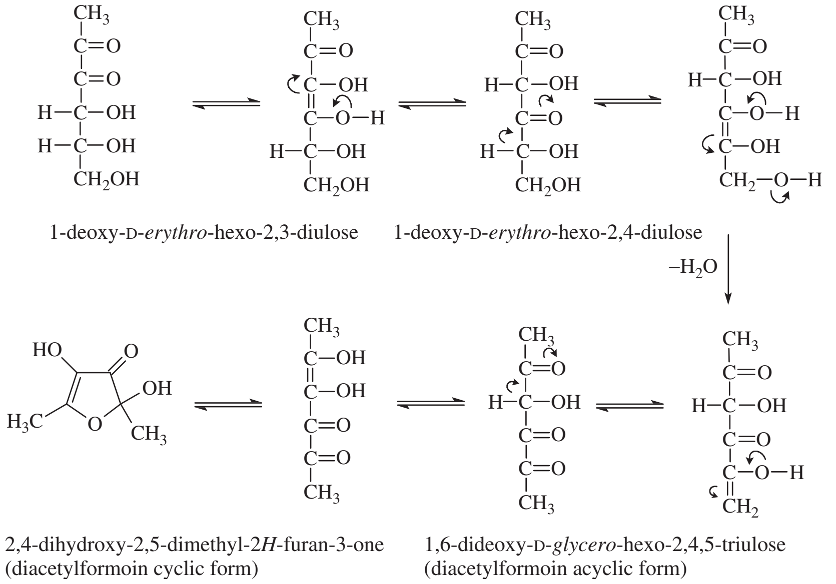


Figure 4.40 Formation of cyclic and acyclic forms of diacetylformoin.

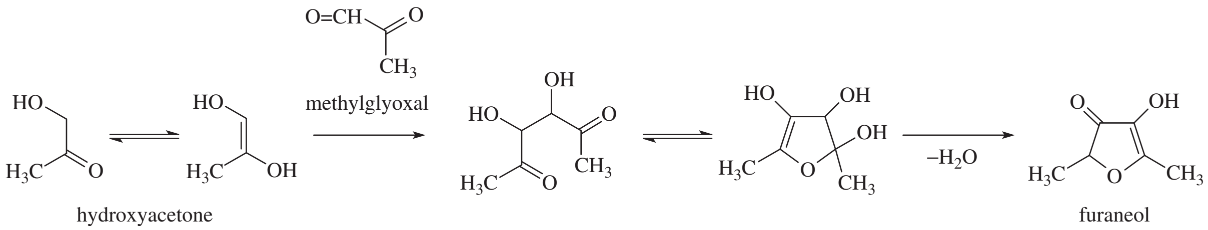


Figure 4.41 Formation of furaneol from methylglyoxal and hydroxyacetone.

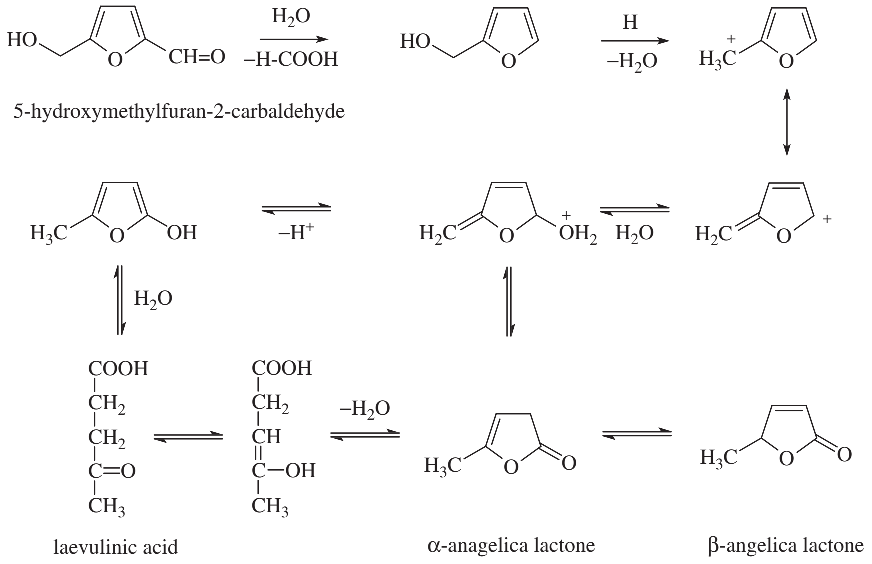


Figure 4.42 Degradation of 5-hydroxymethylfuran-2-carbaldehyde.

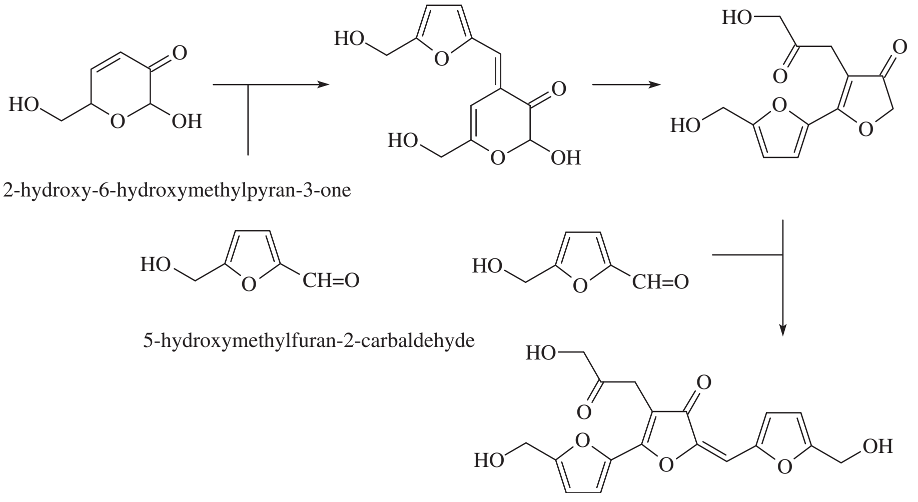
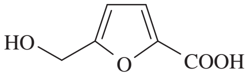
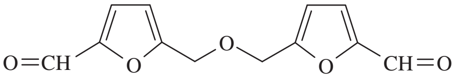


Figure 4.43 Condensation of 5-hydroxymethylfuran-2-carbaldehyde and 2-hydroxy-6-hydroxymethylpyran-3-one.

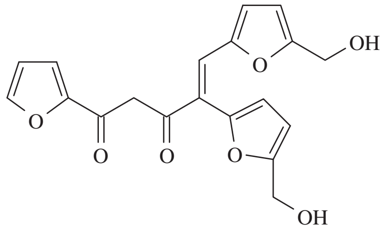


4-198, 5-hydroxymethylfuran-2-carboxylic acid

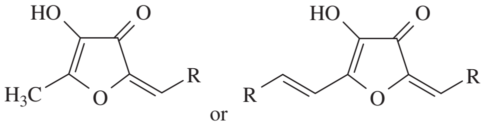


4-199, 5-hydroxymethylfuran-2-carbaldehyde ether

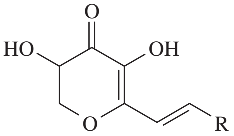
of two molecules of this aldehyde form the corresponding ether (**4-199**) and condensation with 3,4-dideoxy-D-*glycero*-hex-3-en-2-ulose (2-hydroxy-6-hydroxymethylpyran-3-one) yields the corresponding dimer and other compounds (Figure 4.43). The dehydration product of fructose 2-hydroxyacetylfuran gives a coloured trimer with furan-2-carbaldehyde by aldolisation (**4-200**). Condensation products are also formed in reactions of 4-hydroxy-5-methyl-2*H*-furan-3-one (**4-201**) or 3,5-dihydroxy-2-methyl-4,5-



4-200, condensation product of 5-hydroxymethylfuran -2-carbaldehyde with aldehydes



4-201, condensation product of 4-hydroxy-5-methyl-2*H*-furan-3-one with aldehydes



4-202, condensation product of 5,6-dihydro-3,5-dihydroxy-2-methyl-4*H*-pyran-4-one with aldehydes

dihydropyran-3-one (**4-202**) with aliphatic aldehydes and similar products arise with other furans and pyrans. Some of these products are coloured, and uncoloured compounds produce coloured polymers by condensation reactions.

4.7.1.1.5 Formation of reductones

Some of the degradation products of 1-deoxyhexo-2,3-diuloses are reductones, compounds that have the enediol group adjacent to a carbonyl group. In solutions of pH < 6, reductones (like enediolates) occur as monoanions (Figure 4.44) stabilised by resonance. In media of higher pH value, reductones form unstable dianions. In foods, reductones act as antioxidants as in acidic solutions they can reduce a number of organic compounds and metal ions. Their oxidation yields triuloses (Figure 4.45).

Reductones with six carbon atoms in the molecule, formed in acidic solutions, include aliphatic compounds that form as intermediates in the transformation of 1-deoxy-D-*erythro*-hexo-2,3-diulose (Figure 4.37). Diacetylformoin is also one such reductone

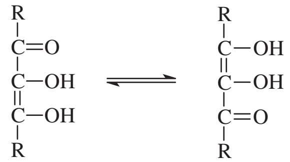


Figure 4.44 Structure of reductones.

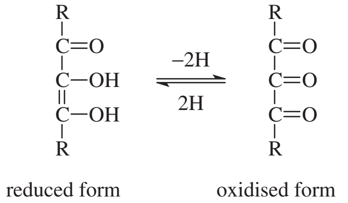


Figure 4.45 Oxidation and reduction of reductones.

(Figure 4.40); another naturally occurring reductone is L-ascorbic acid. In alkaline media, aliphatic reductones with four and three carbon atoms in the molecule, such as *C*-methyltriosoreductone and alicyclic triosoreductone, form by fragmentation of sugars. Reductones containing nitrogen in the molecule are formed as products of the Maillard reaction.

4.7.1.2 Reactions in basic media

4.7.1.2.1 Isomerisation

Enolisation of acyclic forms of reducing monosaccharides occurring in neutral and weakly alkaline media proceeds by a similar mechanism as to their dehydration in acidic solutions. Enolisation of D-glucose or D-fructose produces 1-ene-1,2-diol (enediol anion species 1-ene-1,2-diolates). Enolisation is a reversible reaction, therefore D-glucose, its epimer D-mannose and D-fructose produce a mixture of all three sugars as the main products (Figure 4.46). For example, an aqueous solution of D-glucose of pH 10 is still dominated by D-glucose after 10 days of heating to 35 °C and contains 63.5% D-glucose, 31% D-fructose and 2.5% D-mannose. Isomerisation of glucose to fructose is also known as **aldose–ketose isomerisation** and the change of configuration on carbon C-2 is

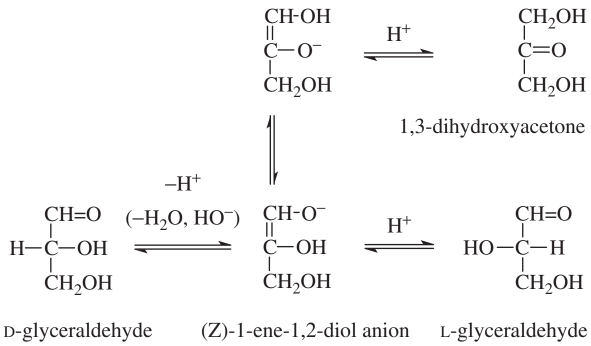


Figure 4.47 Isomerisation of trioses.

called **epimerisation** or **aldose–aldose** isomerisation. The corresponding pairs of aldoses (e.g. glucose and mannose) are called **epimers**. D-Fructose further produces a small amount of D-psicose by 2,3-enolisation. Enolisation can continue along the chain and a small amount of D-sorbose is produced by 3,4-enolisation of fructose, while glucose can yield traces of D-allose and D-mannose gives rise to small amounts of D-altrose.

Isomerisation of pentoses, tetroses, trioses as well as isomerisation of disaccharides takes place analogously. For example, D-ribose produces ketose D-ribulose as the major product and a smaller amount of aldose D-arabinose, glyceraldehyde yields ketose 1,3-dihydroxyacetone (Figure 4.47), the major product of disaccharide lactose is lactulose, which is accompanied by smaller amount of epilactose.

4.7.1.2.2 Rearrangement to acids

When sugars are heated in the solid state (caramelisation) or in alkaline solutions, enolisation precedes the parallel irreversible

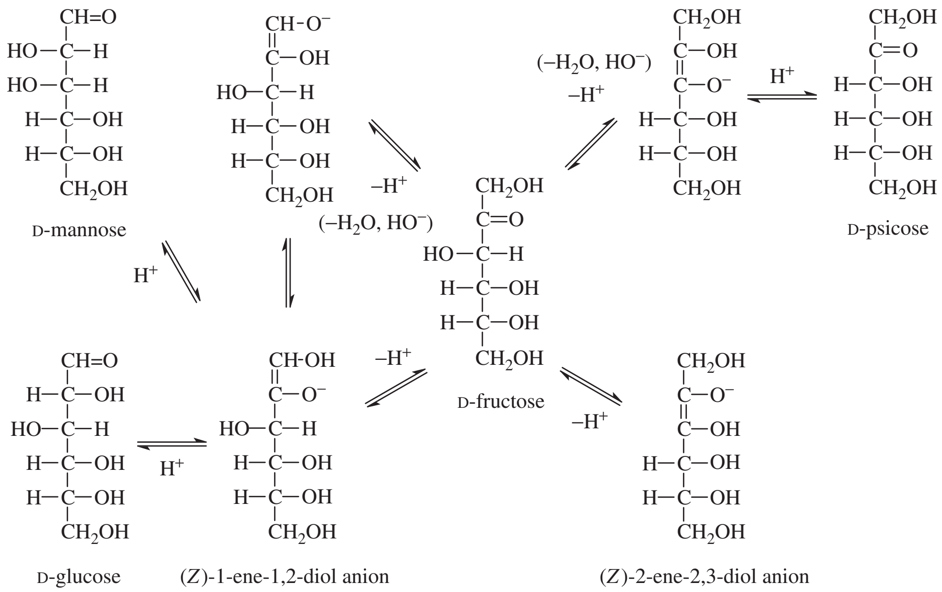


Figure 4.46 Isomerisation of glucose.

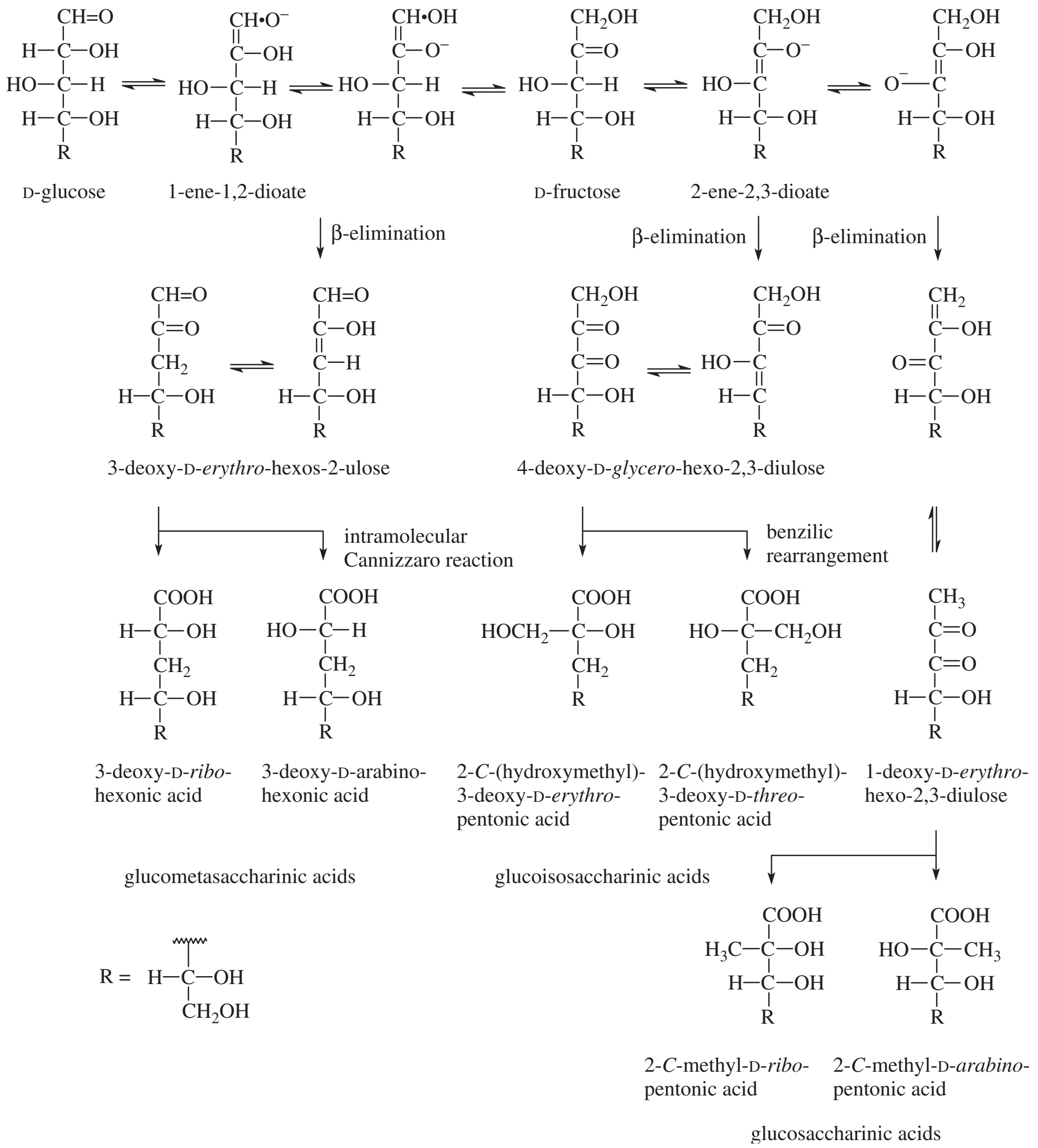


Figure 4.48 Formation of saccharinic acids from glucose and fructose.

rearrangement to sugar acids. The mechanism of this reaction is, using the example of D-glucose and D-fructose, shown in Figure 4.48. The 1-ene-1,2-diolate eliminates the hydroxyl group at C-3 producing the reactive α-dicarbonyl compound (or 2-oxoaldehyde) 3-deoxy-D-*erythro*-hexos-2-ulose. Isomers of **metasaccharinic acids**, such as 3-deoxy-D-*ribo*-hexonic acid (3-deoxy-D-gluconic acid) and 3-deoxy-D-*arabino*-hexonic acid (3-deoxy-D-mannonic acid), are formed from 3-deoxy-D-*erythro*-hexos-2-ulose by intramolecular Cannizzaro reaction. Branched acids, called **isosaccharinic acids**, such as 2-*C*-(hydroxymethyl)-3-deoxy-D-*erythro*-pentonic acid and 2-*C*-(hydroxymethyl)-3-deoxy-D-*threo*-pentonic acid, and also isomers of saccharinic (aldonic) acids (2-*C*-methyl-D-*ribo*-pentonic and 2-*C*-methyl-D-*arabino*-pentonic acids), are formed via 2-ene-2,3-diols by benzilic acid rearrangement of α-dicarbonyl intermediates (2,3-diuloses).

The reaction is generally acid–base catalysed and small amounts of saccharinic acids (their lactones) are formed even in an acidic solution. D-Glucose is the precursor, via 3-deoxy-D-gluconic acid, of the corresponding γ-lactone (Figure 4.31). In the case of 3-*O*-substituted D-glucose, 4-*O*-substituted D-glucose and 1-*O*-substituted D-fructose (and the corresponding disaccharides), the reaction mechanism is analogous and the reaction products are again isosaccharinic and saccharinic acids. The formation of isosaccharinic acids from lactose is shown in Figure 4.49 as an example.

The reaction of trioses takes place in a similar manner (Figure 4.50). D-Glyceraldehyde or 1,3-dihydroxyacetone dehydration yields 1,2-diulose methylglyoxal (also known as pyruvic acid aldehyde or pyruvaldehyde) and the intramolecular Cannizzaro reaction of methylglyoxal yields lactic acid (racemate).

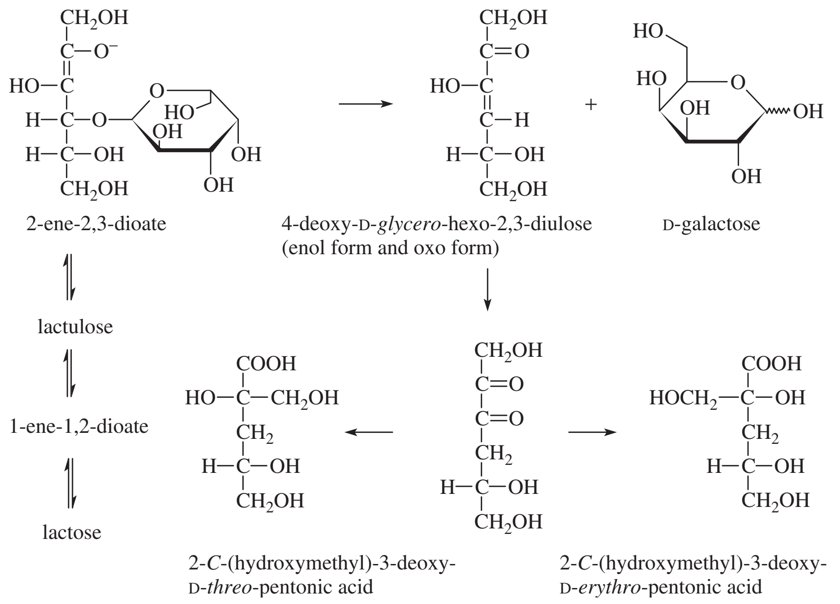


Figure 4.49 Formation of isosaccharinic acids from lactose.

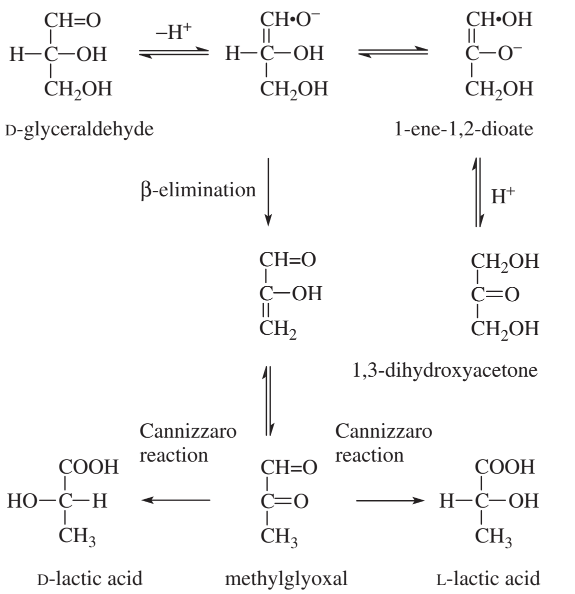


Figure 4.50 Formation of methylglyoxal and lactic acid from trioses.

4.7.1.2.3 Fragmentation and reactions of fragments

Fragmentation

Another important reaction in alkaline media is monosaccharide splitting into fragments with fewer carbon atoms. The cleavage occurs by oxidation of the molecule (after previous isomerisation and dehydration) or by retroaldolisation (opposite reaction to aldolisation). The main products are acids and highly reactive carbonyl, hydroxycarbonyl and dicarbonyl compounds, from which a large number of secondary products result from subsequent reactions such as isomerisation, aldolisation, Cannizzaro reaction and other reactions. Some of these products may be the final reaction products, while others subsequently enter into other reactions, for example with amino acids or proteins.

An example of a degradation product of sugars with one carbon atom in the molecule is formaldehyde. Acetaldehyde, glyoxal and glycolaldehyde have two carbon atoms, while glyceraldehyde, 1,3-dihydroxyacetone (1,3-dihydroxypropan-2-one), hydroxyacetone (1-hydroxypropan-2-one and also known as acetol), lactaldehyde (2-hydroxypropanal or lactic acid aldehyde), methylglyoxal (pyruvaldehyde) and hydroxymethylglyoxal are all examples of products with three carbon atoms. The four carbon fragment is hydroxybiacetyl (1-hydroxybutan-2,3-dione) and aldose D-erythrose, and the five carbon atom fragments are pentoses, such as D-arabinose. At the same time, aliphatic three and four carbon reductones are produced, such as the so-called triosoreductone, *C*-methyltriosoreductone and alicyclic reductones. Monosaccharides produce more fragments than disaccharides, and glucose gives more fragments than fructose. Fragmentation of monosaccharides is illustrated by the fragmentation of D-glucose (Figure 4.51) and D-fructose (Figure 4.52).

Formation of carboxylic acids

Fragmentation of sugars and glycosuloses leads not only to reactive α-dicarbonyl and α-hydroxycarbonyl products, but also to carboxylic acids. Carboxylic acids with short chains can be formed by several mechanisms. Acetic acid is a major degradation product of sugars, especially in neutral and alkaline solutions. As a product of degradation of hexoses, acetic acid is almost exclusively produced from C-1/C-2 atoms of their transformation products, 1-deoxy-2,3-diuloses. The most common pathway is the isomerisation of the reactive α-dicarbonyl intermediate to

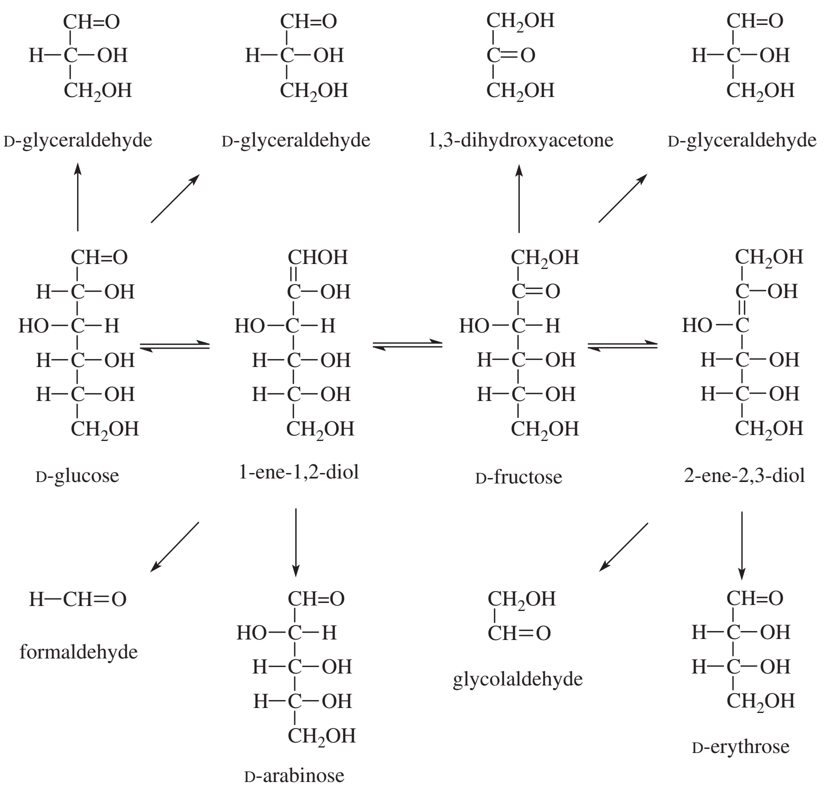


Figure 4.51 Fragmentation of glucose and fructose.

β-dicarbonyl compounds, followed by hydrolytic (β-dicarbonyl) cleavage (reverse Claisen condensation). For example, 1-deoxy-D-*erythro*-hexo-2,3-diulose, via 1-deoxy-D-*erythro*-hexo-2,4-diulose and after nucleophilic attack of hydroxyl anion to the C-2 carbonyl group and subsequent protonisation of C-4 carbonyl group, yields acetic acid and an 1-ene-1,2-diol intermediate, which isomerises to yield either C_4 ketose D-erythrulose or C_4 aldoses D-erythrose and D-threose (Figure 4.53). Oxidative (α-dicarbonyl) cleavage of 1-deoxy-D-*erythro*-hexo-2,4-diulose yields acetic acid and D-tetronic acids (D-erythronic and D-threonic acids). Hydrolytic cleavage of 1-deoxy-D-*erythro*-hexo-2,4-diulose leads to D-glyceric acid and hydroxyacetone (acetol). Oxidative cleavage of the 1-ene-1,2-diol intermediate leads to the formation of D-*glycero*-tetros-2-ulose and dehydration of the 1-ene-1,2-diol yields 3-deoxytetros-2-ulose and (after isomerisation) 1-deoxytetro-2,3-diulose. Another possibility is the formation of acetic acid from carbons C-3/C-4 of 1-deoxy-D-*erythro*-hexo-2,4-diulose by hydrolytic cleavage of 1-deoxytetro-2,3-diulose (Figure 4.54). To a lesser extent acetic acid results from carbons C-5/C-6 after dehydration of 1-deoxy-D-*erythro*-hexo-2,4-diulose to diacetylformoin and its hydrolytic β-cleavage.

Similar mechanisms, cleavage of C-1/C-2 bonds of 1-amino-1,4-dideoxyhexo-2,3-diulose, lead to the formation of formic acid and 3-deoxypentos-2-ulose. 1-Amino-1,4-dideoxy-D-*glycero*-hexo-2,3-diulose arises almost exclusively by transformation of oligosaccharides, such as maltose, linked by a (1→4) glycosidic bond and hydrolysis of the bound amino compound yields 1,4-dideoxy-D-*glycero*-hexo-2,3-diulose (see Section 4.7.5.3.3).

In addition to formic, acetic and glyceric acids, the transformation of sugars yields a number of other acids, from C_2 (glycolic acid) to C_6 acids. Acids are formed at higher temperatures, especially in alkaline media and in the presence of oxygen. To a lesser extent, acids may arise from α-dicarbonyl intermediates by oxidative cleavage. When oxygen binds to one of the carbonyl group carbons, electron transfer (Baeyer–Villiger rearrangement) and hydrolysis of the product yield two molecules of carboxylic acids (Figure 4.55). The options also include oxidation of the terminal aldehyde groups of sugars by molecular oxygen, which produces carboxylic acids occurring in acidic solutions in the form of lactones.

Reactions of fragments with water

Carbonyl compounds with electron-withdrawing groups on C_α carbon can be hydrated in aqueous solution. Carbonyl compounds and their hydrates are in equilibrium with each other and most hydrates (as well as hemiacetals) are known to decompose spontaneously to the corresponding carbonyl compounds (Figure 4.56). For example, formaldehyde in dilute aqueous solutions is almost completely hydrated, acetaldehyde is hydrated to about 60%, while higher (fatty) aldehydes and acetone are not hydrated at all. The hydration of carbonyl compounds is the result of the positive inductive effect of alkyl substituents. In addition to the hydrate (methyleneglycol), concentrated solutions of formaldehyde also contain a series of oligomeric hydrates of general formula H–(O–CH_2)$_n$–OH. In neutral and especially in acidic

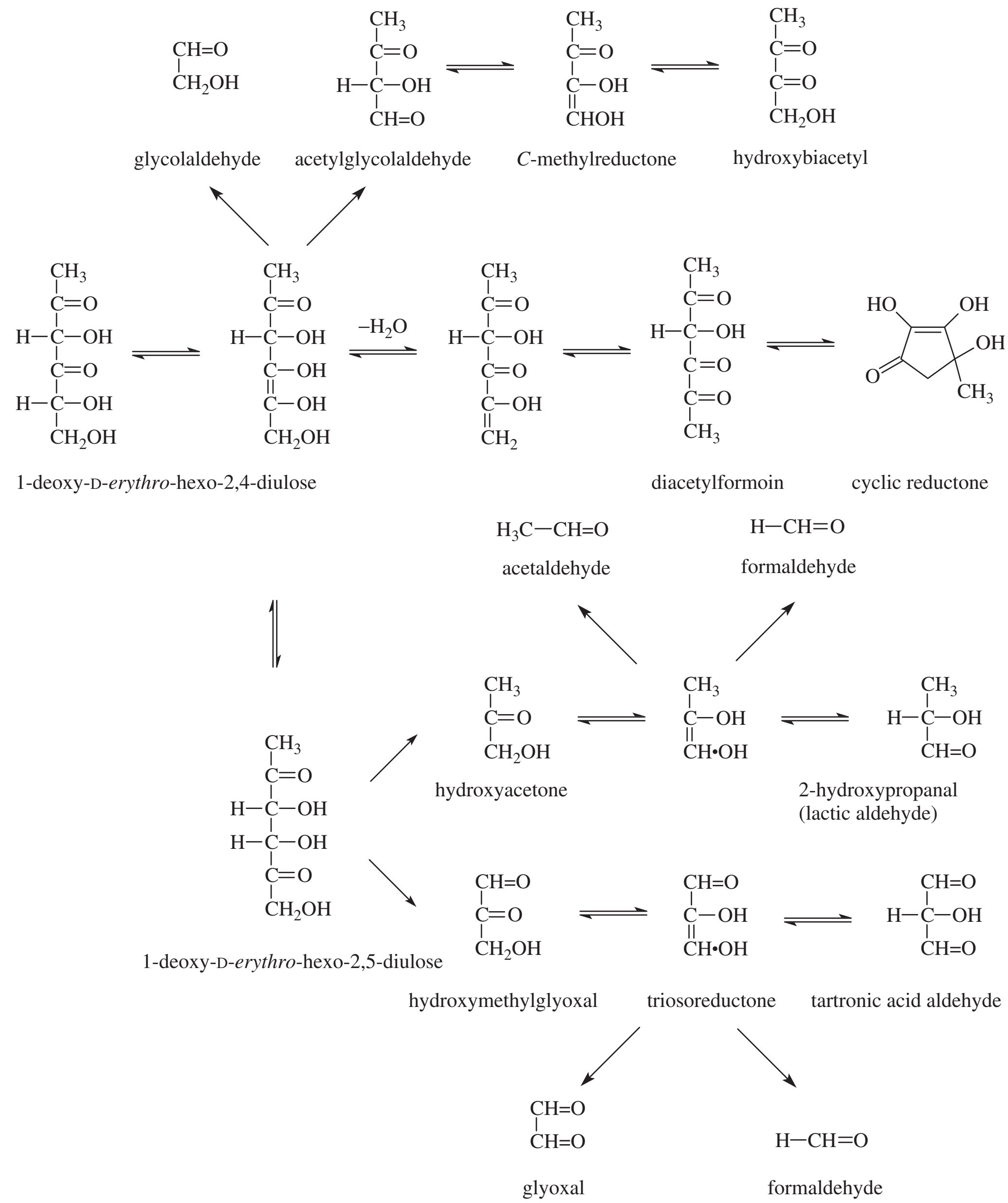


Figure 4.52 Formation of fragments and reductones from fructose 2,3-enolisation products.

solutions, formaldehyde also forms a cyclic trimer, 1,3,5-trioxan, and polymeric hydrates known as paraformaldehyde (Figure 4.57). A cyclic trimer, 2,4,6-trimethyl-1,3,5-trioxan (paraldehyde, **4-203**), is formed in acidic solutions from acetaldehyde.

The product of microbial transformation of glycerol, 3-hydroxypropionaldehyde, undergoes a reversible dimerisation and hydration (Figure 4.58), which results in an equilibrium of 3-hydroxypropionaldehyde, 1,1,3-trihydroxypropane (3-hydroxypropionaldehyde hydrate) and 2-(2-hydroxyethyl)-4-hydroxy-1,3-dioxane (3-hydroxypropionaldehyde dimer). Hydrates also form from α-hydroxycarbonyl compounds, such as glycolaldehyde, glyceraldehyde and 1,3-dihydroxyacetone, and α-dicarbonyl compounds, such as glyoxal, methylglyoxal and related substances (Figure 4.59). These hydrates are spontaneously transformed into various cyclic dimers with 1,4-dioxolane rings. Hydrates similarly form some higher α-hydroxycarbonyl and α-dicarbonyl compounds, such as L-dehydroascorbic acid (Figure 5.27) and 2,3-dioxo-L-gulonic acid.

The hydrates of most carbonyl compounds, such as methylglyoxal hydrate (Figure 4.60), are unstable and exist only in solutions. Hydrates that are stable in the solid state arise, for example, from (*E*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose. Oligomers that are stable in the solid state are also formed from hydrates of glycolaldehyde (**4-204**) or glyoxal (**4-205**) and thermal degradation of these hydrates produces a number of different products. For example, glyoxal hydrate trimer produces the corresponding dimer, monomer,

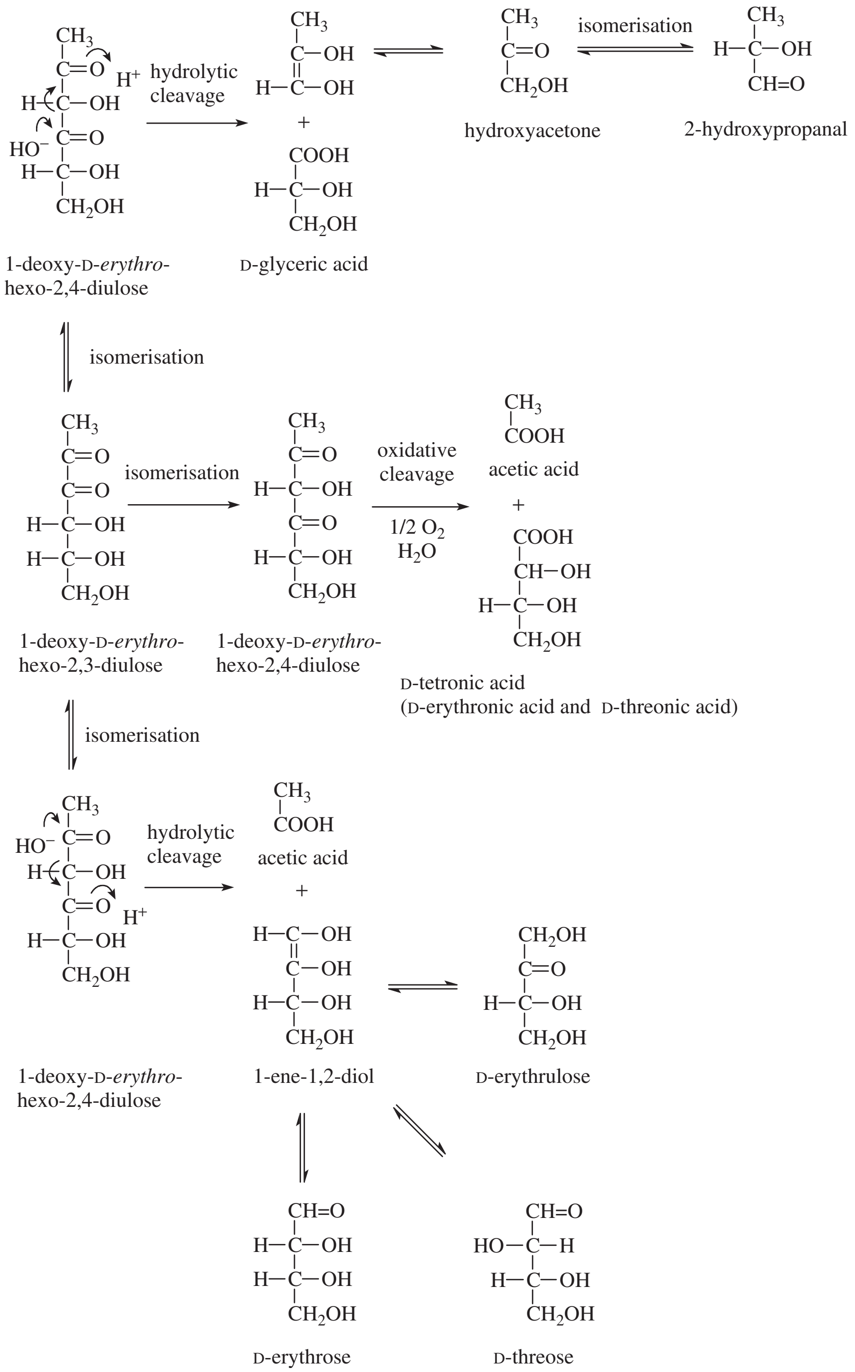


Figure 4.53 Formation of acetic acid, glyceric acid and other products from 1-deoxy-D-*erythro*-hexo-2,3-diulose.

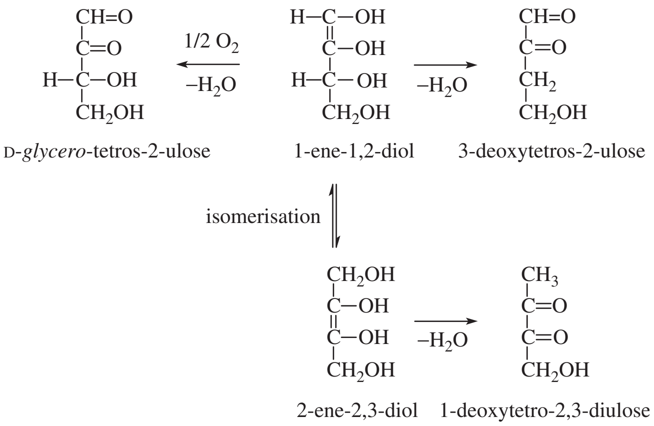
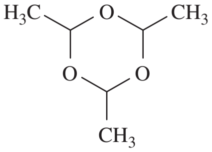
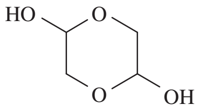


Figure 4.54 Isomerisation and other reactions of 1-ene-1,2-diol derived from 1-deoxy-D-*erythro*-hexo-2,3-diulose.



4-203, 2,4,6-trimethyl-1,3,5-trioxan



4-204, glycolaldehyde hydrate dimer

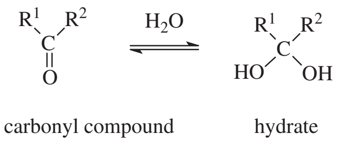


Figure 4.56 Addition of water and formation of a hydrate.

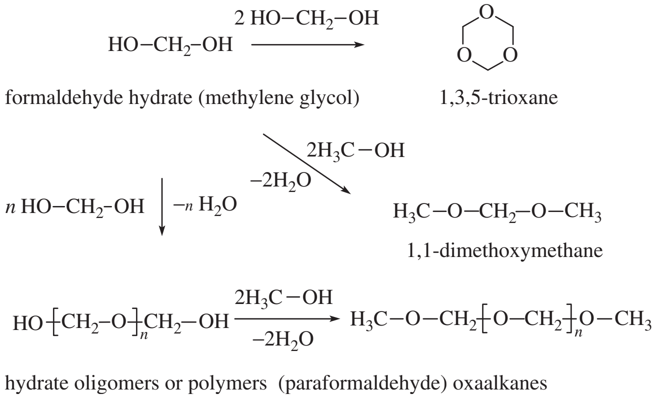
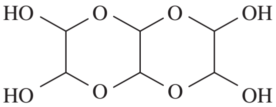


Figure 4.57 Reactions of formaldehyde hydrate.

carbon dioxide and formic acid[9] when heated to 250 °C. Methylglyoxal hydrate yields about 40 different compounds during heating to 100 °C. The main decomposition products are acetic acid (30%), propane-1,2-diol (18%), hydroxyacetone (acetol, 7%) and 2,4-dimethyl-1,3-dioxolane, which is a cyclic acetal of formaldehyde and propane-1,2-diol (7%). Other products are formed in smaller quantities, for example 2-hydroxypropanal (lactic aldehyde) and formic acid. Dehydration of hydroxyacetone yields acrolein (Figure 4.60).



4-205, glyoxal hydrate trimer

[9]Formic acid is a common degradation product of sugars and also the oxidation product of formaldehyde. It is effective as a specific reagent for reductive amination of imminium ions to give secondary amines in the Maillard reaction. The reaction is known as a Wallach or Leuckart reaction (reactions of formates or formamide): $R^1R^2N^+{=}CH{-}R + H{-}COOH \rightarrow R^1R^2N{-}CH_2{-}R + CO_2$.

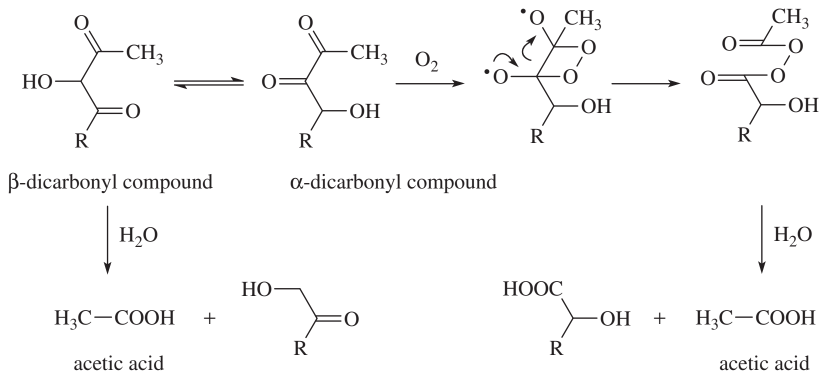


Figure 4.55 Formation of acetic acid by cleavage of α- and β-dicarbonyl compounds.

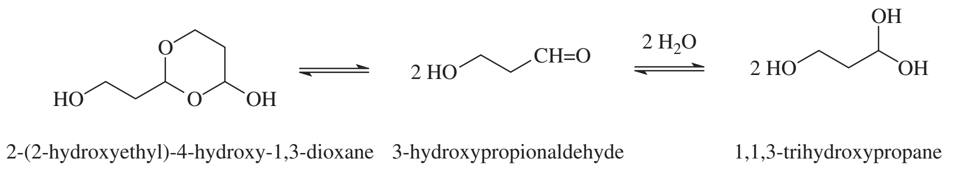


Figure 4.58 Formation of 3-hydroxypropionaldehyde hydrate and dimer.

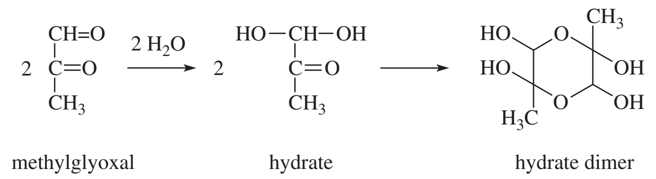


Figure 4.59 Formation of methylglyoxal hydrate and hydrate dimer.

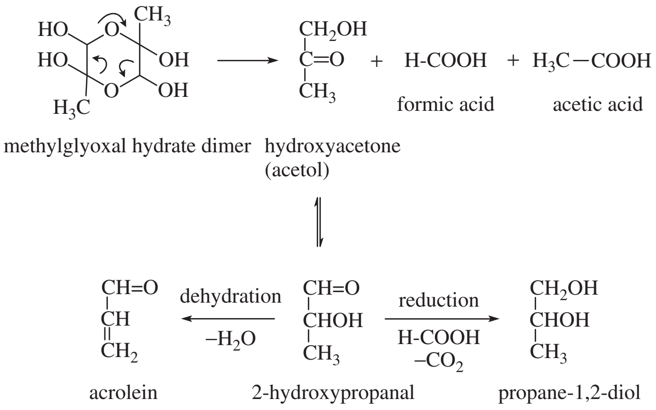


Figure 4.60 Degradation of methylglyoxal and hydroxyacetone.

Other reactions of fragments

Sugar fragments isomerise in alkaline solutions just like the original sugars. Glyceraldehyde, for example, isomerises to 1,3-dihydroxyacetone (Figure 4.47), hydroxyacetone isomerises to lactic aldehyde, hydroxymethylglyoxal to triosoreductone and *C*-methyltriosereductone to 3-oxobutanal (Figure 4.52).

Sugar fragments can also be rearranged into acids. For example, dehydration of glyceraldehyde and 1,3-dihydroxyacetone yields methylglyoxal, which is rearranged to lactic acid (Figure 4.50). Aldehydes and ketones that have at least one hydrogen on the C_α carbon undergo aldolisation in alkaline solutions. The reaction of formaldehyde with the reactive degradation sugar product glycolaldehyde yields glyceraldehyde that isomerises to 1,3-dihydroxyacetone and then reacts, in the rate-determining step, with another molecule of formaldehyde. The reaction of formaldehyde in alkaline earth hydroxide solutions, leading to a mixture of optically inactive sugars (so-called formose), is called the formose reaction. Originally it was assumed that glycolaldehyde, which catalyses this reaction, arises from formaldehyde. It was later shown that formaldehyde contained trace amounts of glycolaldehyde as an impurity. The formed tetroses break down relatively quickly by retroaldolisation to glycolaldehyde, and the cycle is repeated. In subsequent steps, tetroses can react with formaldehyde or to yield pentoses to hexoses with branched or straight chains. At the level of hexoses, the reaction virtually stops because hexoses are relatively stable compounds that react slowly with formaldehyde (Figure 4.61).

The main aldolisation products from the reaction of glycolaldehyde with D-glyceraldehyde are D-arabinose and D-xylose. Similarly, D-glyceraldehyde and 1,3-dihydroxyacetone aldolisation produce a mixture of D-fructose and D-sorbose. The reaction is highly stereoselective as a diol arrangement of *threo*-configuration and not of *erythro*-configuration results on carbons C-3 and C-4. A similar highly selective aldol reaction of 1,3-dihydroxyacetone phosphate is catalysed by the enzyme aldolase. Branched sugar D-dendroketose is formed as one of the minor products.

The mutual condensation of some degradation products of sugars yields important alicyclic substances called cyclopentenolones, which are characterised by a caramel flavour similar to maltol, and other secondary reaction products of sugars. For example, condensation of hydroxyacetone with lactic aldehyde yields the basic member of the homologous series 2-hydroxy-3-methylcyclopent-2-en-1-one, which is known as cyclotene (Figure 4.62). In aqueous solutions, cyclotene exists partly as the corresponding hydrate.

Condensation of sugar degradation products in alkaline, neutral and weakly acidic media or a sequence of isomerisation, dehydration and oxidation/reduction reactions of sugars can lead to aromatic compounds such as phenols. For example, minor degradation products of D-glucose and D-fructose in neutral media are catechol and pyrogallol (**4-206**), but products with a higher number of carbon atoms can also be formed, such as 3,4-dihydroxybenzaldehyde, 3,4-dihydroxyacetophenone (**4-207**) and 3-hydroxy-6-hydroxymethylchrom-2-one (**4-208**). Phenolic compounds play an particularly important role in the non-enzymatic browning reactions of cellulose and cellulose products, which results in yellowing of paper and cotton textile products, because phenolic compounds are easily oxidised to coloured quinones that further react with the original phenols or amino compounds with the formation of coloured reaction products. Quinones also react with sugars and may form *C*-glycosides (**4-209**). During thermal processing of foods, *C*-glycosides are produced with reducing sugars, as are flavonoids such as flavan-3-ols (catechins). Reaction of maltose with (+)-catechin yields, for example, (+)-catechin-6-*C*-β-D-glucopyranosyl-(4→1)-*O*-α-D-glucopyranose (**4-210**). Carbonyl and dicarbonyl sugar transformation products form addition compounds with tannins and other naturally occurring phenolic substances even under physiological

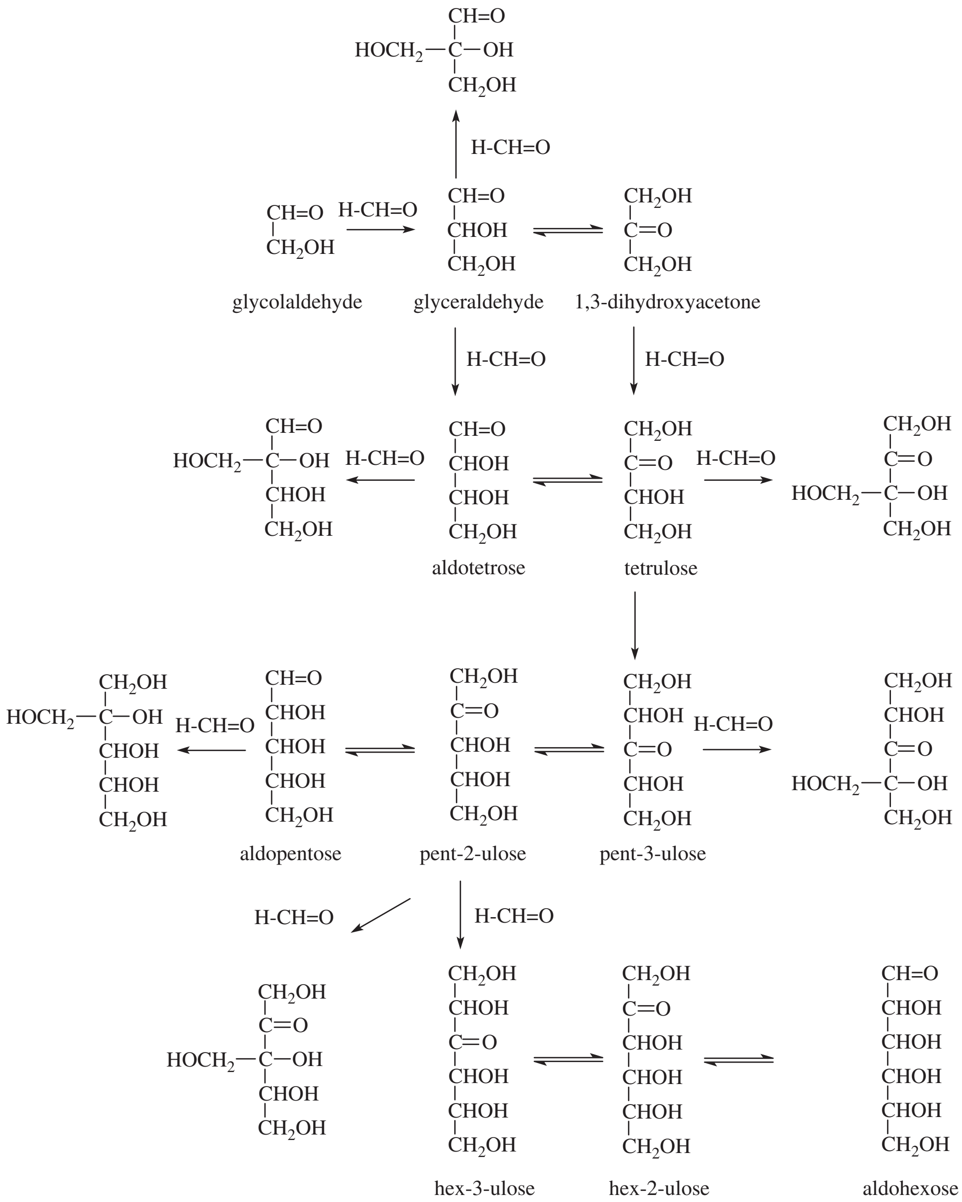
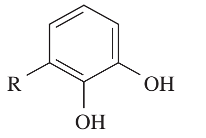
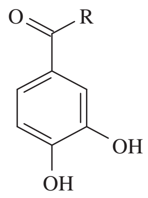


Figure 4.61 Formose reaction.



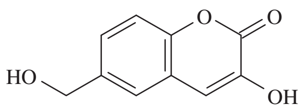
4-206, phenols

pyrocatechol, R = H
pyrogallol, R = OH



4-207, aldehydes and ketones

3,4-dihydroxybenzaldehyde, R = H
3,4-dihydroxyacetofenone, R = CH_3



4-208, 3-hydroxy-6-hydroxymethylchrom-2-one

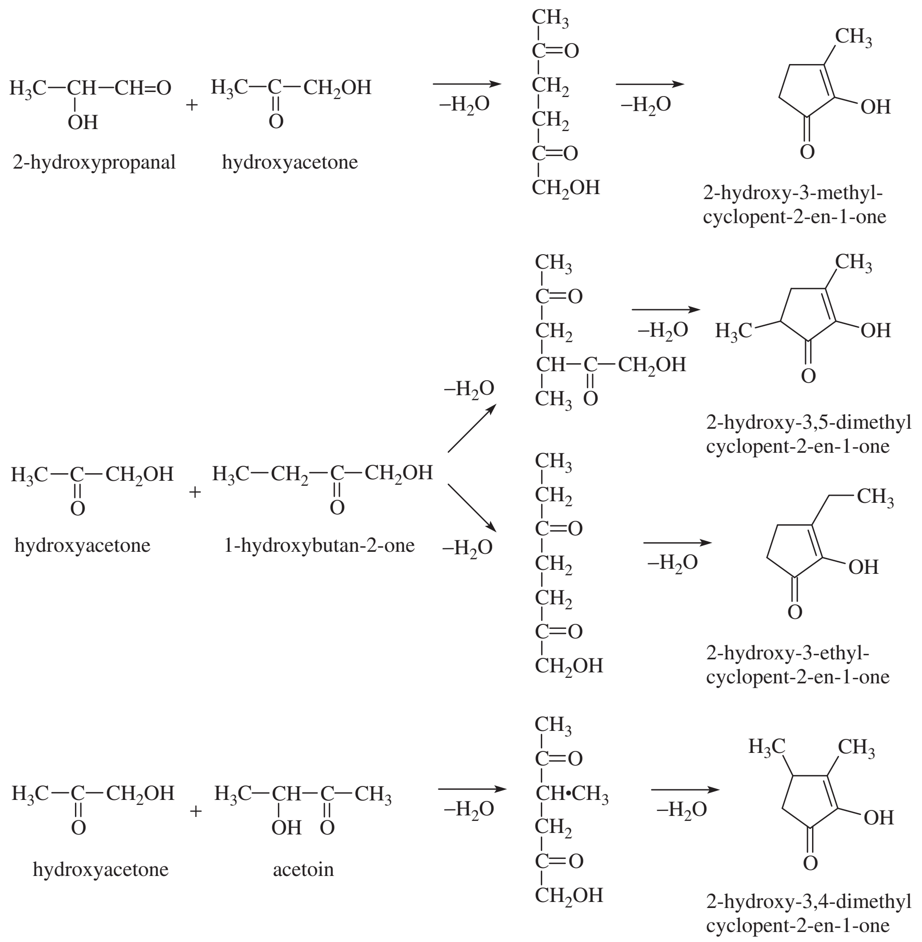
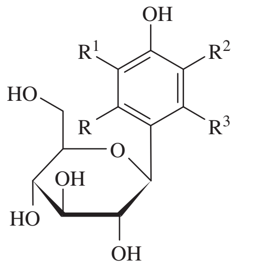
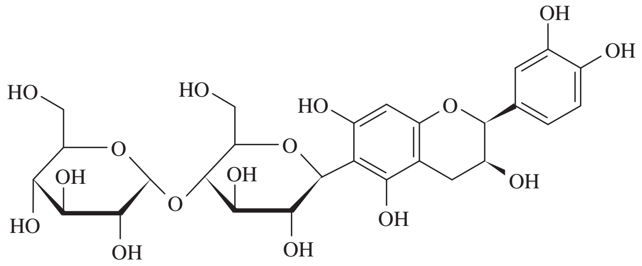


Figure 4.62 Formation of cyclopentenolones.



4-209, *C*-glucosides of phenols



4-210, product of maltose reaction with (+)-epicatechin

conditions. An example is the reaction of the main component of tea leaves (−)-epigallocatechin-gallate (see Section 8.3.6.2.1) with methylglyoxal (Figure 4.63).

4.7.1.3 Oxidations and reductions

4.7.1.3.1 Oxidation by oxygen, peroxides and peroxyl radicals

An aldehyde group is oxidised to a carboxyl group enzymatically during caramelisation and by common oxidising agents in basic and acidic media. The oxidation by atmospheric oxygen, called autoxidation, is slow under neutral conditions and faster in alkaline media. During autoxidation, D-glucose and D-fructose produce unstable hydroperoxides via 1-ene-1,2-diol. Their decomposition yields arabinonic and formic acids (Figure 4.64). Glucose and fructose are also oxidised in the presence of transition metal ions, such as Cu^{2+} and Fe^{3+}, via enediol complex with metal and oxygen, to D-*arabino*-hexos-2-ulose, which can be further oxidised with decomposition and reduction of metal ions.

Similar products are formed by oxidation with hydrogen peroxide in alkaline media. Hydrogen peroxide is also formed by

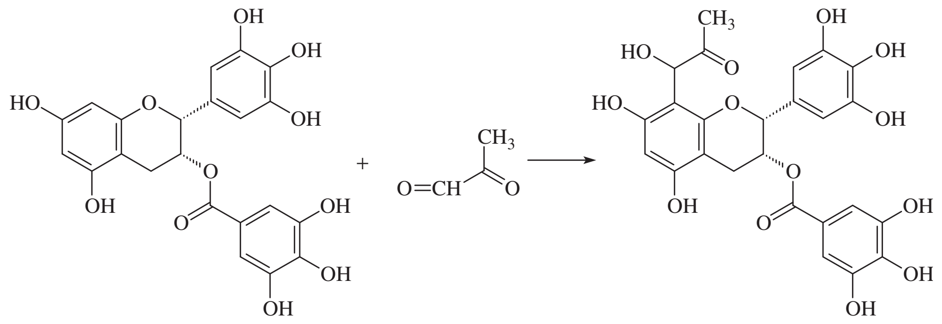


Figure 4.63 Reaction of methylglyoxal with epigallocatechin gallate.

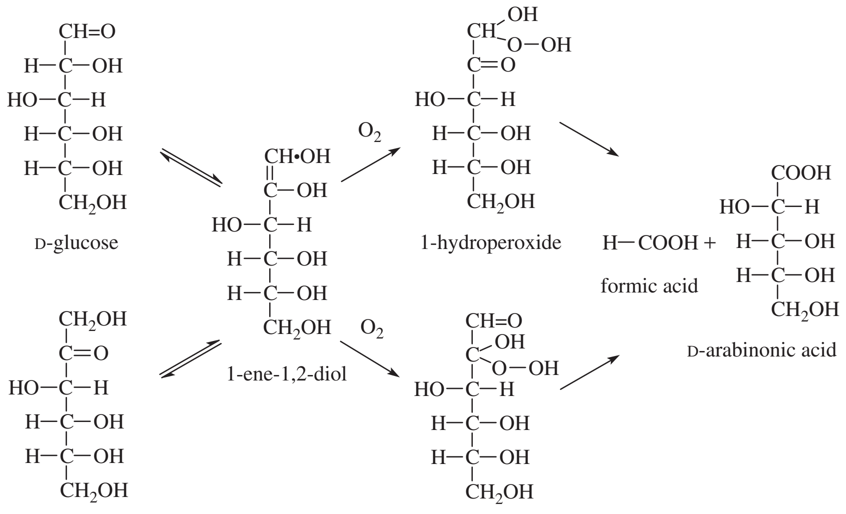


Figure 4.64 Autoxidation of glucose and fructose.

decomposition of the sugar enediol complex with metal and oxygen. In neutral and weakly acidic solutions, hydrogen peroxide itself is a weak oxidising agent. However, in the presence of Fe^{2+} ions, the decomposition of hydrogen peroxide generates free radicals (hydroxyl radicals $^•OH$), which also oxidise sugars to glycosuloses (Figure 4.65).

The mechanism of oxidation by lipid peroxyl radicals involves C–H bond cleavage at carbon C-2. The radical that is produced reacts with oxygen with the formation of a peroxyl radical that spontaneously decomposes to the corresponding glycosulose radical and hydrogen peroxide (Figure 4.66). This hydrogen peroxide radical, after disproportionation to hydrogen peroxide and oxygen ($2\,HOO^• \rightarrow H_2O_2 + O_2$), may act in other oxidation reactions. The formation of a hydrogen peroxide radical ($HOO^•$) by this mechanism, which assumes transfer of hydrogen in the five membered intermediate structure, is not the only possibility for the oxidation of sugar by peroxyl radicals. An alternative reaction is decomposition with hydrogen transfer in the intermediate six-membered structure. This generates lower aldonic acids (such as tetronic acid), hydrogen peroxide and glyoxal (Figure 4.67).

4.7.1.3.2 Cannizzaro reaction

A special case of an oxidation–reduction (redox) reaction catalysed typically by alkalis (in some cases the reaction also takes place in acidic solutions) is a Cannizzaro reaction. It occurs in aldehydes that do not have a hydrogen atom on the C_α carbon, which means that the usual aldol condensation cannot take place. One molecule of aldehyde is oxidised with the simultaneous reduction of the second molecule and the reaction product is a mixture of acid and primary alcohol (Figure 4.68). A Cannizzaro reaction of formaldehyde yields formic acid and methanol; furan-2-carbaldehyde gives rise to furan-2-carboxylic acid (also known as 2-furoic acid or pyromucic acid) and 2-furfuryl alcohol (2-furylmethanol or 2-hydroxymethylfuran).

Formaldehyde, as the most reactive aldehyde, undergoes Cannizzaro reaction even with aldehydes that have a hydrogen atom at the C_α carbon. Formaldehyde also reacts to some extent with aldoses that are reduced to sugar alcohols (alditols) and formaldehyde is oxidised to formic acid (Figure 4.69). Reaction of formaldehyde with D-glucose yields D-glucitol, reaction with D-glyceraldehyde

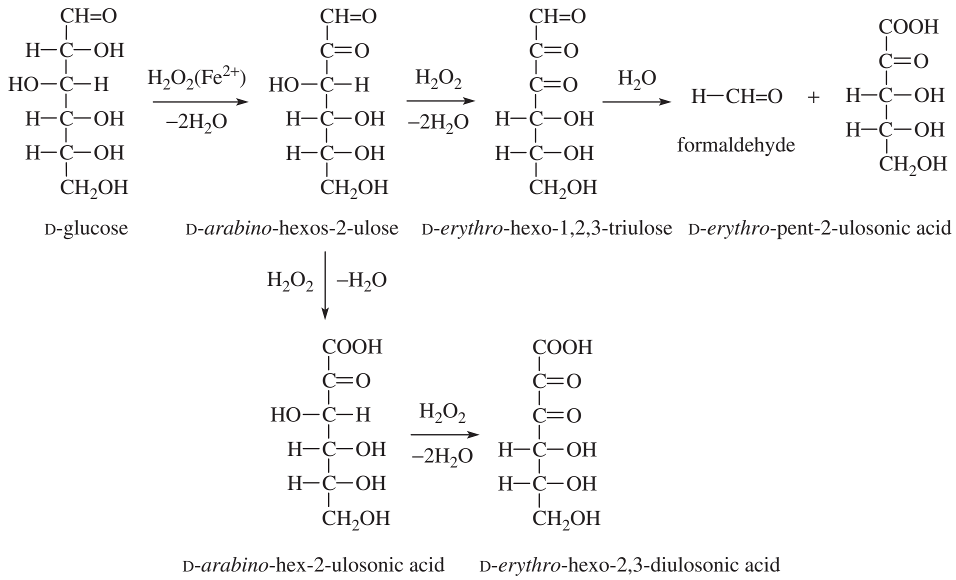


Figure 4.65 Oxidation of glucose to glycosulosonic acids by hydrogen peroxide in the presence of Fe^{2+} ions.

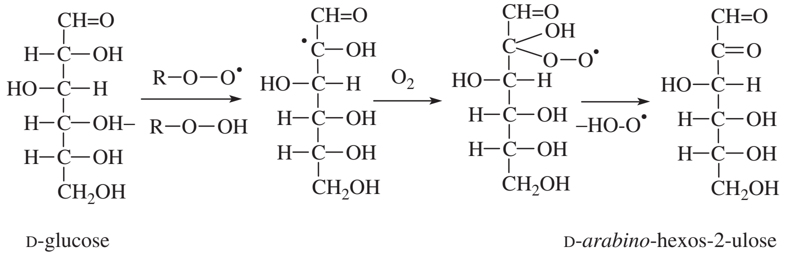


Figure 4.66 Oxidation of glucose to glycosulose by peroxyl radicals.

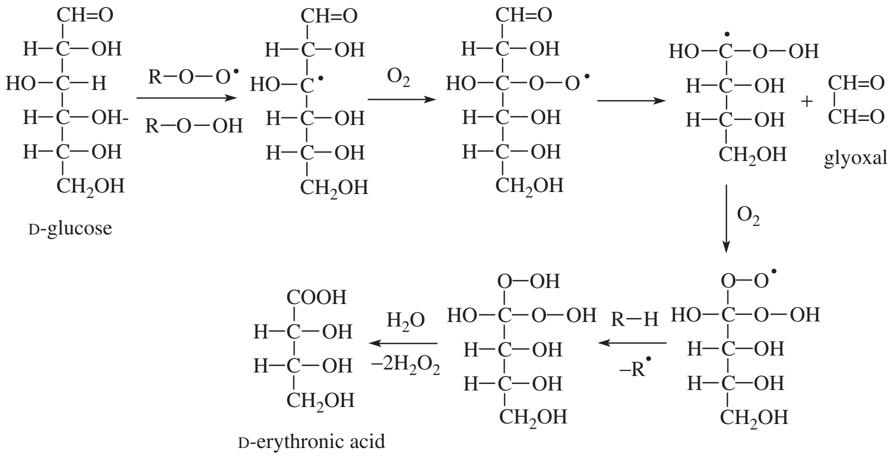


Figure 4.67 Oxidation of glucose to aldonic acid and glyoxal by peroxyl radicals.

yields glycerol and reaction with glycolaldehyde gives ethane-1,2-diol (ethylene glycol).

With glyoxal, methylglyoxal and other α-dicarbonyl compounds, the Cannizzaro reaction is an intramolecular redox reaction, which yields 2-hydroxy acids (Figure 4.70). Glycolic acid results from glyoxal and lactic acid is produced from methylglyoxal.

4.7.1.3.3 Disproportionation of α-hydroxycarbonyl and α-dicarbonyl compounds

Enediol forms of α-hydroxycarbonyl compounds react with α-dicarbonyl compounds through interconversion, in which α-hydroxycarbonyl compounds are transformed into α-dicarbonyl compounds and vice versa (Figure 4.71).

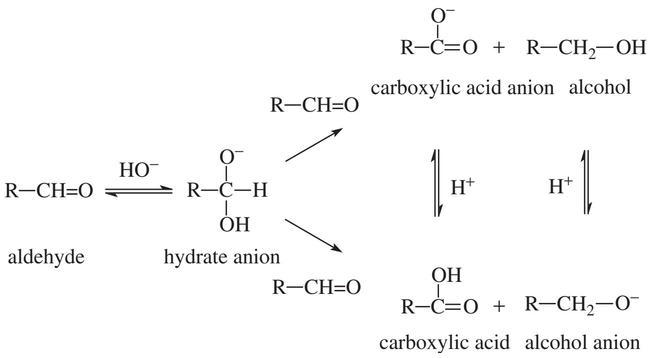


Figure 4.68 General mechanism of Cannizzaro reaction.

4.7.1.3.4 *Disproportionation of α-hydroxycarbonyl and α-dicarbonyl compounds*

Enediol forms of α-hydroxycarbonyl compounds react with α-dicarbonyl compounds with interconversion, in which α-hydroxycarbonyl compounds are transformed into α-dicarbonyl compounds and vice versa (Figure 4.66). The reaction explains a number of oxidation–reduction reactions that occur in degradation products of sugars and in the Maillard reaction. The transfer of two protons takes place within the complex of the α-dicarbonyl compound, with the endiol in either the basic energy singlet state or in the excited triplet state, through the more advantageous biradical mechanism.

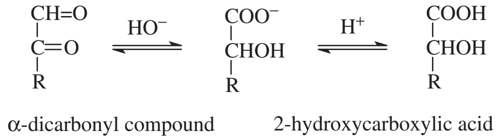


Figure 4.70 Cannizzaro reaction of α-dicarbonyl compounds.

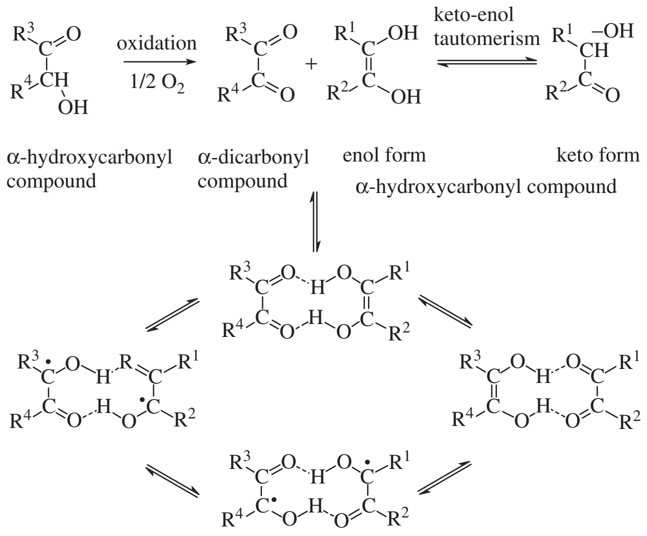


Figure 4.71 Disproportionation of α-hydroxycarbonyl and α-dicarbonyl compounds.

4.7.2 Derivatives of monosaccharides

The chemical reactions of polyols are similar to reactions of the hydroxyl groups of monosaccharides. For example, glycerol yields a mixture of different products containing diglycerol, oligoglycerols and polyglycerols. Alditols can dehydrate to form monoanhydro and dianhydro derivatives in acidic solutions. Acid catalysed dehydration of D-glucitol yields a mixture of cyclic anhydrides that are used as emulsifiers. Oxidation of the secondary alcoholic group of D-glucitol on C-2 or C-5 by the microorganisms *Acetobacter xylinum* or *Gluconobacter oxydans* produces L-sorbose, an intermediate used in the synthesis of L-ascorbic acid. Alditols, as well as cyclitols, are virtually stable during food storage and processing.

4.7.3 Oligosaccharides

The properties and reactivity of reducing oligosaccharides are comparable to the properties and reactivity of monosaccharides, non-reducing oligosaccharides behave and react similarly to glycosides of monosaccharides.

Acids hydrolyse oligosaccharides to monosaccharides under simultaneous intramolecular dehydration that produces small amounts of anhydrosugars as by-products. The rate of hydrolysis (inversion) depends on the acidity and temperature, but also on other factors related to the structure of the oligosaccharides, such as inductive and steric effects. The hydrolysis of α-anomers of disaccharides is easier than hydrolysis of β-anomers, pyranosides are more stable than furanosides and non-reducing oligosaccharides are more stable than the reducing sugars. The mechanism of hydrolysis of most disaccharides is similar to the mechanism of hydrolysis of *O*-glycosides. The hydrolysis of higher sugars takes place via disaccharides and other lower oligosaccharides. The α- and β-glycosidic bonds are also cleaved enzymatically under the action of α- and β-glycosidases, similarly to the case of heteroglycosides.

The opposite reaction to hydrolysis (inversion) is reversion, when di- and higher oligosaccharides are synthesised from

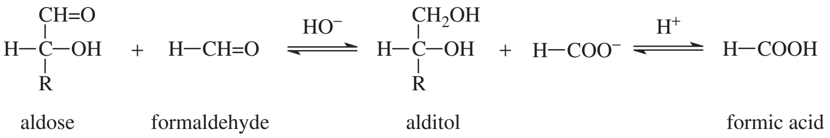


Figure 4.69 Cannizzaro reaction of formaldehyde with aldoses.

monosaccharides and lower oligosaccharides in acid solutions. Reversion often proceeds together with acid catalysed hydrolysis and the glycosidic bonds can isomerise simultaneously, so that, *O*-β-D-oligosaccharides are transformed into *O*-α-D-oligosaccharides and vice versa.

During heating in solution, fructooligosaccharides are less stable than glucooligosaccharides. For example, fructose decomposes already at 60 °C in neutral solutions, while glucose or saccharose solutions can be rapidly heated to temperatures up to 100 °C without decomposition. Hydrolysis of saccharose are undesirable reactions in the production of saccharose from sugar beet or sugar cane, which depends on the presence of hydrolases, pH, temperature, concentration of saccharose and other factors. Invertases, which catalyse saccharose hydrolysis during the diffusion process, have to be inactivated by heating the raw juice to temperatures up to 80 °C (see Section 4.4.2.1.2). At this temperature and with the relatively low pH of the juice (pH 5.2–5.8), however, chemical hydrolysis can cause significant loss of saccharose (e.g. in solutions of pH 5.2 and also at pH 11.4, saccharose loss is about 0.5% per hour) that can be prevented by an increase in pH through the addition of calcium hydroxide, as the rate of hydrolysis of saccharose is minimal in solutions of pH around 8.4. Alkaline pH, on the other hand, initiates non-enzymatic browning reactions of reducing monosaccharides. The main manifestation of these reactions is the formation of yellow, brown to black pigments called melanoidins, which, along with the pigments (known as melanins) derived from phenolic compounds in enzymatic browning reactions, cause discolouration of raw sugar and molasses.

Reducing oligosaccharides are somewhat more stable than monosaccharides in alkaline solutions. Under similar conditions as those for monosaccharides, isomerisation takes place of the sugar unit bound at the reducing end of the oligosaccharide, which leads to the formation of the corresponding epimer (isomerisation of the type aldose–aldose) and bound aldoses isomerise to bound ketoses (isomerisation of the type aldose–ketose).

4.7.4 Polysaccharides

Functional properties of polysaccharides (formation of viscous dispersions and gels) are associated with the mutual interactions of their chains and interactions with other food components (especially water, proteins and lipids).

The most important reaction of polysaccharides is hydrolysis. Hydrolysis by digestive enzymes of the digestive system or by colon microbial saccharases allows many polysaccharides, and especially starch, to be utilised. Spontaneous hydrolysis of polysaccharides by endogenous enzymes of food raw materials or microbial saccharases is an important reaction in many food technologies. It is related to the technological, functional and organoleptic characteristics of a number of food products (including production of bread, post-mortem changes in meat, fruit ripening and other processes). Hydrolysis of some polysaccharides (e.g. starch and inulin) is used to obtain monosaccharides, oligosaccharides and polysaccharide functional derivatives. Derivatives of some polysaccharides (e.g. starch and cellulose) are used as food additives (hydrocolloids).

4.7.5 Maillard reaction

Some of the most important and widespread chemical reactions occurring during storage and processing of food are reactions of reducing sugars with amino compounds. These reactions produce a number of highly reactive carbonyl compounds that react with each other and also with the amino compounds present. This series of reactions is generally known as the Maillard reaction,[10] the consequence of which is the formation of brown pigments called **melanoidins** and therefore these reactions belong to the group of **non-enzymatic browning reactions**. In addition to the Maillard browning reaction, two other major types of non-enzymatic browning reactions are recognised:

- **caramelisation**
- **reactions of proteins with oxidised lipids**.

The Maillard reaction can therefore be regarded as a special case of non-enzymatic browning reactions of sugars with proteins (amino acids).

Only a small number of compounds produced in these reactions have been characterised. These are mainly low molecular weight compounds, which are relatively stable during isolation and identification. Much less is known about the reactive intermediates that arise in very low concentrations and, moreover, are usually decomposed during isolation. Very little is known about the resulting free radicals. Knowledge of the structure of these compounds is very important because they play an important role in the formation of flavour-active substances, high molecular weight coloured pigments (melanoidins) and also in the reactions that occur *in vivo*.

The attention of food chemists has mainly been focused on the following.

- Formation of a brown colour, which may either be desirable as a manifestation of the Maillard reaction (such as the colour of bread crust, roasted coffee and fried onions) or may be a negative phenomenon (e.g. in the production of dried foods, especially milk, as well as fruits and vegetables).
- Formation of aromatic compounds and aromatic compounds with adverse organoleptic properties.
- Nutritional and physiological aspects of the Maillard reaction (reduction of the nutritional value of foods mainly due to the reaction of sugars and other carbonyl compounds with lysine, an essential and often limiting amino acid).
- Antioxidant activities of the reaction products (mainly reductones and coloured melanoidins).

[10]The reaction was named after the French chemist Louis Camille Maillard, who first described the formation of brown pigments formed during heating of glucose with glycine. The Maillard reaction has always attracted, and still attracts, the attention of many chemists. It has been studied for a century by food chemists, nutritionists, physiologists and physicians, but it is still impossible to present a complete reaction scheme due to its complexity.

- Toxicity of some products, especially mutagenic and carcinogenic substances.

The most important food saccharides involved in the Maillard reaction are the monosaccharides glucose and fructose, and in some cases ribose (e.g. in meat and meat products). The most important disaccharides are lactose (in milk and dairy products) and maltose (in cereal products, e.g.in malt). Saccharides linked by glycosidic bonds in glycoproteins, glycolipids, heteroglycosides and non-reducing sugars (such as saccharose) can participate in the Maillard reaction after hydrolysis to the parent monosaccharides.

Reaction partners of reducing sugars are mainly proteins and amino acids. Proteins react with reducing carbohydrates primarily through the ε-amino group of bound lysine. To a smaller extent α-amino groups of *N*-terminal amino acids and other amino acid functional groups, such as the mercapto group of cysteine and guanidyl group of arginine, are also involved in the Maillard reaction. In addition to proteins and amino acids, biogenic amines also contribute significantly to the Maillard reaction in some foods (e.g. in cheeses).

In addition to sugars and their degradation products and the degradation products of amino acids (amines, ammonia and aldehydes), these reactions also include carbonyl compounds already present in foods as primary substances (e.g. aldehydes and ketones occurring in essential oils), ascorbic acid and carbonyl compounds formed in foods from precursors other than carbohydrates (aldehydes resulting from fat oxidation), thereby the reactions become even more complex.

Owing to the complexity of the Maillard reaction, model systems containing only one reducing sugar and one amino acid are often chosen because they are simpler systems than foods. Research has shown that even in simple reaction systems, for example in glucose and glycine solutions, many tens of reaction products are formed. Therefore, even in such simple systems, the Maillard reaction mechanisms have not been fully elucidated and all the reaction products have still not been identified. The modified reaction schemes according to Hodge and Tressl[11] are given in Figure 4.72 and Figure 4.73, respectively.

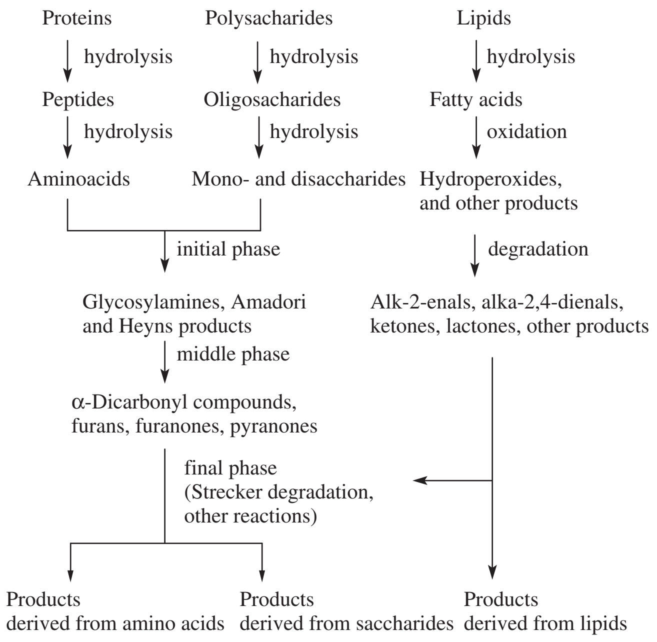


Figure 4.73 Schematic representation of the Maillard reaction according to Tressl.

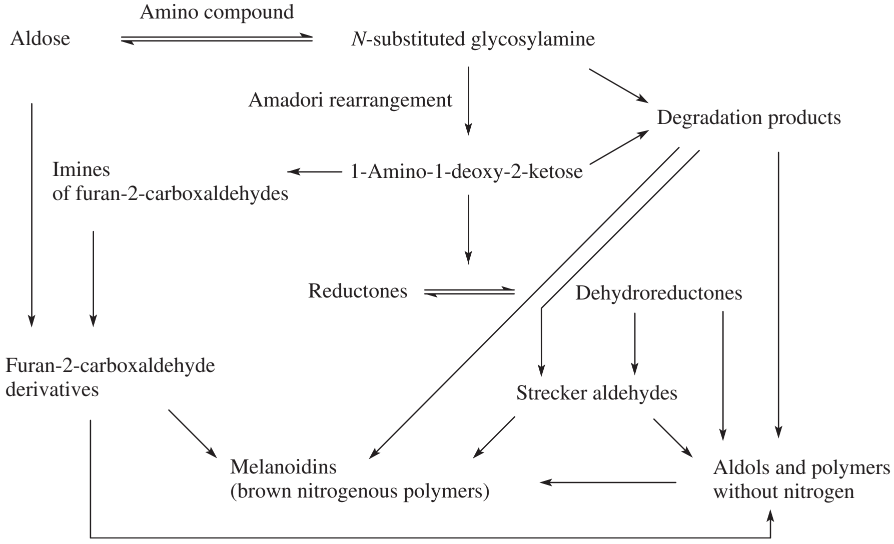


Figure 4.72 Schematic representation of the Maillard reaction according to Hodge.

[11]The classification of individual reactions taking place within the Maillard reaction was first described by John Edward Hodge in 1953, more than 50 years ago, and it is still the most concise description of the Maillard reaction. Exactly 40 years later Roland Tressl supplemented this scheme.

In the reaction of an aldose with an amino compound, such as the reaction of glucose with amino acids, three basic phases are recognised:

- the **initial phase** involves the formation of glycosylamine followed by Amadori rearrangement;
- the **middle phase** involves dehydration and fragmentation of saccharides and Strecker degradation of amino acids;
- the **final phase**, which includes reactions of intermediates leading to the formation of heterocyclic and other compounds, and are usually important as flavour-active compounds and coloured pigments called melanoidins that cause the yellow, brown or black colouration.

Ketoses may also enter into the reaction, and then the formation of glycosylamine is followed by Heyns rearrangement (Section 4.7.5.1). The reaction could also include not only carbonyl compounds derived from the degradation of sugars, but also Strecker aldehydes arising from amino acids and reactive aldehydes and other compounds produced as secondary decomposition products of fatty acid hydroperoxides in lipid peroxidation.

4.7.5.1 Formation of glycosylamines and rearrangement to aminodeoxysugars

The Maillard reaction starts by the addition of a non-protonated amino group of an amino compound (amine, amino acid or protein) to an electron deficient carbon atom of a polarised carbonyl group of a reducing sugar (acyclic form of an aldose or ketose). The resulting addition product, *N*-substituted hemiaminal (formerly also known as monotopic carbinolamine), dehydrates with the formation of an imine (formerly also known as azomethine or a Schiff base). The electron density on the carbonyl group carbon and thus the ease of addition is influenced by polar factors (mainly inductive effects of the carbonyl group substituents) and steric factors. Alkyl and aryl carbonyl group substituents have a positive inductive effect (electron repelling; +I). The most reactive substance is therefore formaldehyde, and less reactive compounds are higher aliphatic aldehydes. The introduction of the electronegative group exhibiting a negative inductive effect (electron attracting; –I) into the aliphatic substituent to a position adjacent to the carbonyl group, such as a hydroxyl group (in sugar) or other carbonyl group (in glycosuloses), decreases the electron density on the carbon of the carbonyl group, which results in a higher reactivity of such a derivative. The reactivity of carbonyl compounds, therefore, generally decreases in the following order:

- aldoses > ketoses
- trioses > tetroses > pentoses > hexoses > disaccharides
- α-dicarbonyl compounds > aldehydes > ketones > saccharides > oxoacids.

The reactivity of sugars is mainly determined by the amounts of acyclic forms present. The reactivity of amino compounds is closely related to their basicity and therefore falls in the following order:

- ammonia > amines > amino acids
- 6-amino acids > . . . > 3-amino acids > 2-amino acids.

Another very important factor influencing the reactivity of both reaction partners is pH. Protonisation of the carbonyl group in acidic solutions increases the reactivity of nucleophilic reagents, while protonised amino groups are less reactive as the nitrogen atom does not have a free electron pair (Figure 4.74). Acid–base properties of amino acids, peptides and proteins (formation of cations, amphions and anions) are described in Section 2.2.3.1.

The mutual reaction of a carbonyl compound with an amino compound is shown in Figure 4.75. The concentration of both reactants varies with pH value of the solution in the opposite direction (cation concentration of the carbonyl compound increases, while the concentration of the unprotonised amino compound decreases with decreasing pH) and the reaction rate reaches a maximum in slightly acidic solutions (e.g. in reactions of aldoses with amines) or in slightly basic solutions (in reactions of α-dicarbonyl compounds with amino acids). Schiff bases are usually unstable compounds that are stabilised by subsequent reactions. The exception is the Schiff base of an aromatic aldehyde with an aromatic amine, in which case the reaction equilibrium is shifted significantly in favour of the Schiff base. The Schiff bases formed from sugars are stabilised by a reversible cyclisation to *N*-substituted glycosylamines (Figure 4.76).

In solutions, *N*-glycosylamines (as well as the parent sugars) occur mainly in the form of pyranoses or furanoses. They mutarotate as a result of the formation of an immonium intermediate (Schiff base cation) and are easily hydrolysed to the original sugar and amine (Figure 4.77). Glycosylamines derived from aromatic or heterocyclic amines are relatively stable, while glycosylamines derived from aliphatic amines and especially glycosylamines derived from amino acids (that is, glycosylamino acids) are subjected to further reactions to give more stable products.

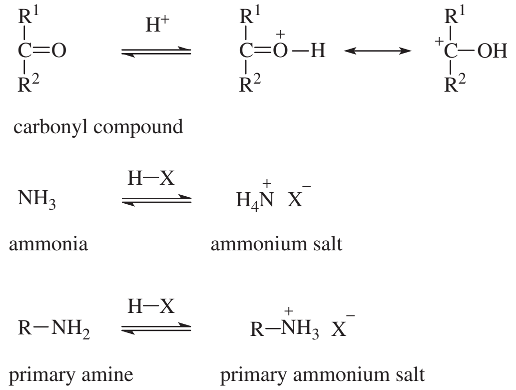


Figure 4.74 Formation of cations of carbonyl compounds and amino compounds.

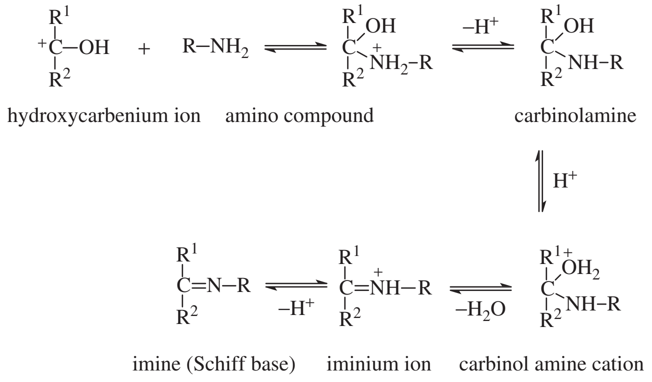


Figure 4.75 General scheme of reactions of carbonyl compounds with amino compounds.

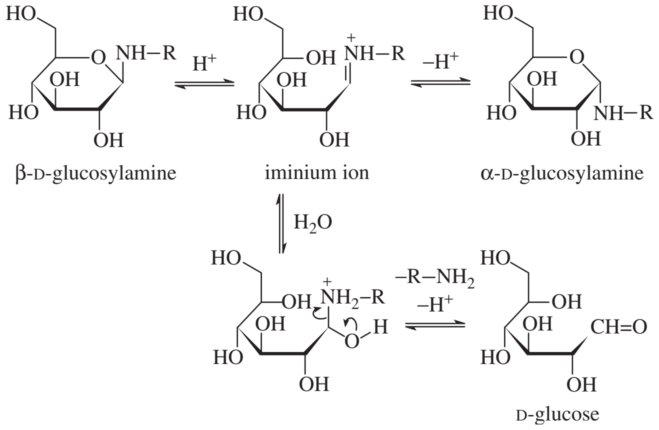


Figure 4.77 Mutarotation and hydrolysis of glycosylamines.

The fate of *N*-glycosylamines depends on water activity, pH and temperature. In media with moderate to high water content and a pH range of 5–7, the Schiff bases are usually rearranged to substantially more stable compounds. Aldosylamines are transformed into **ketosamines**, also known as **1-amino-1-deoxyketoses** or **Amadori compounds**, by the **Amadori rearrangement**. Ketosylamines are similarly transformed into **aldosamines** (**2-amino-2-deoxyaldoses**, also called **Heyns compounds**) by the **Heyns rearrangement**. The reaction is equivalent to the aldose–ketose isomerisation. The Amadori and Heyns rearrangements are generally acid–base catalysed reactions (Figure 4.78).

The Amadori rearrangement begins by protonisation of aldosylamine, leading to ring opening to form a semiacetal immonium ion. This reaction is followed by proton cleavage on carbon C-2 and the enaminol produced stabilises to the keto form by isomerisation and by cyclisation to form the hemiacetal. The reaction rate is significantly influenced by the form in which the glycosylamines occur. Aldosylamines present as furanoses rearrange about ten times more quickly than pyranoses. The reacting amino compound acts simultaneously as a catalyst, because the amino acid carboxyl group provides the necessary proton. The reaction rate increases in the presence of carboxylic acids and phosphates. The catalytic effect of carboxylic acids is explained by reaction of the immonium ion with the carboxyl anion to form an intermediate, which decays to enaminol and carboxylic acid (Figure 4.79).

The active ion of phosphates (the catalytic effect is optimal at pH 5–7) is the dihydrogenphosphate anion. This anion is a base that withdraws a proton from the carbon C-2 of glycosylamine during the Amadori rearrangement. The Amadori and Heyns rearrangements are favoured under acidic conditions, but these reactions may also take place in a strongly alkaline medium, as they are generally acid–base catalysed (Figure 4.80). The Amadori rearrangement has long been considered to be an irreversible reaction. Some studies in recent years, however, have admitted the

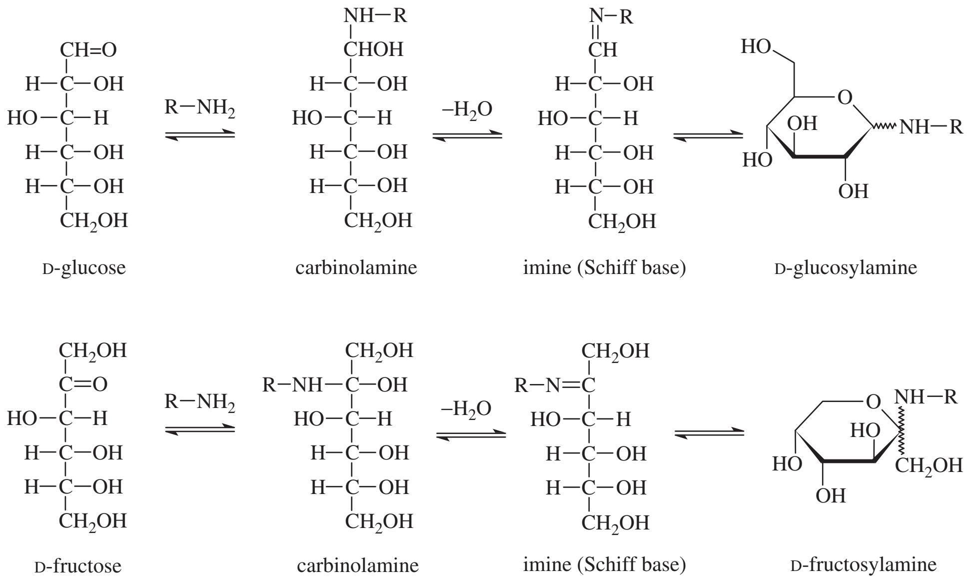
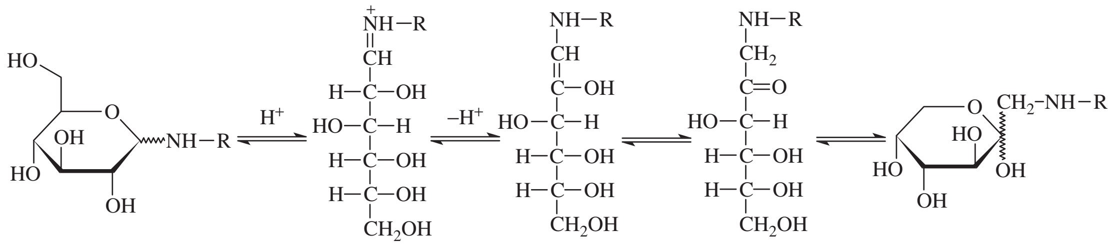
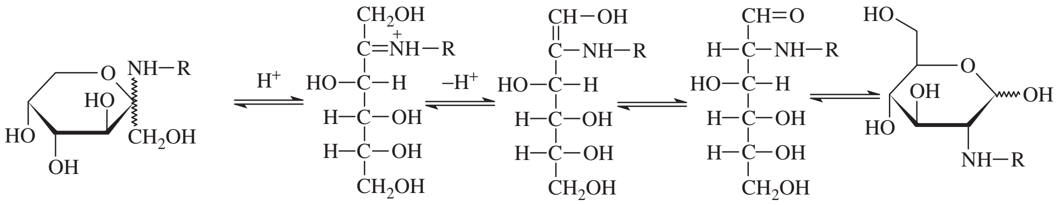


Figure 4.76 Formation of glycosylamines.



D-glucosylamine iminium ion enol form oxo form cyclic form

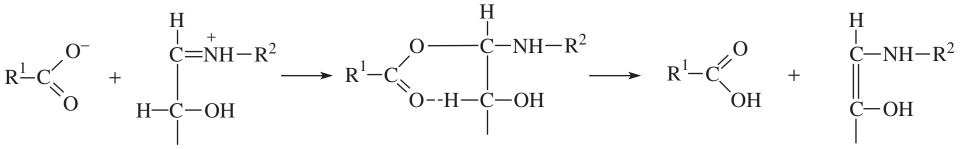
D-fructosamine (1-amino-1-deoxy-D-fructose)



D-fructosylamine iminium ion enol form oxo form cyclic form

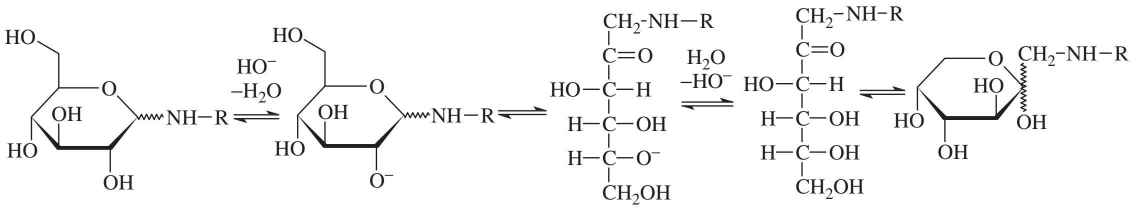
D-glucosamine (2-amino-2-deoxy-D-glucose)

Figure 4.78 Amadori and Heyns rearrangements catalysed by acids.



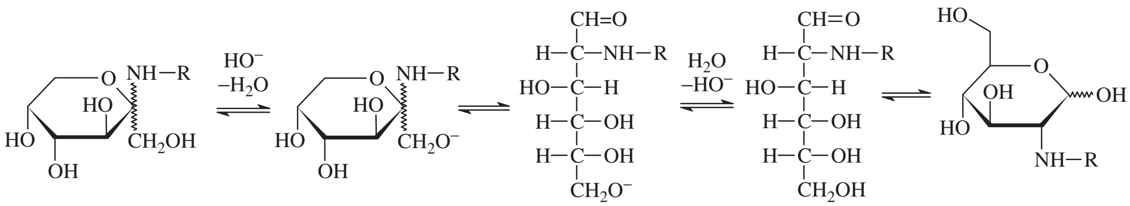
carboxylate ion iminiumion carboxylic acid enamine enol form

Figure 4.79 Mechanism of Amadori rearrangement catalysed by carboxylic acids.



D-glucosylamine oxo form cyclic form

D-fructosamine (1-amino-1-deoxy-D-fructose)



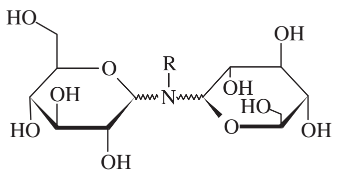
D-fructosylamine oxo form cyclic form

D-glucosamine (2-amino-2-deoxy-D-glucose)

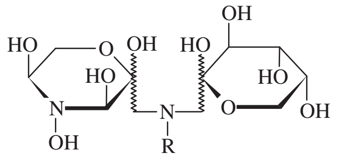
Figure 4.80 Amadori and Heyns rearrangements catalysed by hydroxyl ions.

possibility of a reversible reactions to form the parent sugar or its epimer.

Ketosamines have been demonstrated in a number of stored and heat-processed foods, such as dried fruits, dried vegetables, dried milk, soy sauces and other products. They arise similarly in infusions containing glucose and amino acids and even in the human organism (particularly in diabetics) as intermediates of the Maillard reactions that are on-going *in vivo*. Some aminodeoxysugars occurring in foods, however, arise by biochemical (enzymatic) reactions, for example D-glucosamine and D-galactosamine that are the building units of chitin.



4-211, di-D-glucosylamine



4-212, di-D-fructosamine

4.7.5.2 Other reactions of glycosylamines

The Amadori and Heyns rearrangements are the main but not the only possible conversions of *N*-glycosylamines. Aldosylamines derived from primary amines (and also from amino acids) can react with another aldose molecule to form a dialdosylamine before the Amador rearrangement. For example, D-glucose reaction with D-glucosylamine gives di-D-glucosylamine (**4-211**). Analogously to aldosylamines, dialdosylamines are rearranged to diketosamines and di-D-fructosamine (**4-212**) is produced from di-D-glucosylamine.

In foods or parts of foods with low water activity, other transformation pathways can also occur. In alkaline solutions and at low temperatures and even during pyrolysis, transamination can yield the corresponding oxoacid and 1-amino-1-deoxyalditol. In weakly acidic or neutral solutions and at elevated temperatures, Schiff bases can form cyclic products. Schiff bases derived from amino acids can yield oxazolidinone derivatives; Schiff bases derived from peptides generate imidazolidinone derivatives. These cyclic products decarboxylate easily to form isomeric imines (Figure 4.81). The Strecker degradation of bound amino acids in Schiff bases derived from sugars and amino acids yields the corresponding aldehyde, carbon dioxide and 2-deoxyaldose through oxidation.

Schiff bases derived from α-hydroxycarbonyl fragmentation products of sugars (such as glycolaldehyde), where the free hydroxyl group in the original glycolaldehyde is in the β-position to the nitrogen atom (in *N*-glycosylamines it is usually part of a pyranose or a furanose ring, therefore their decomposition via an Amadori product prevails) may yield imine by rearrangement of an azomethine ylid. This imine is a decarboxylated analogue of an

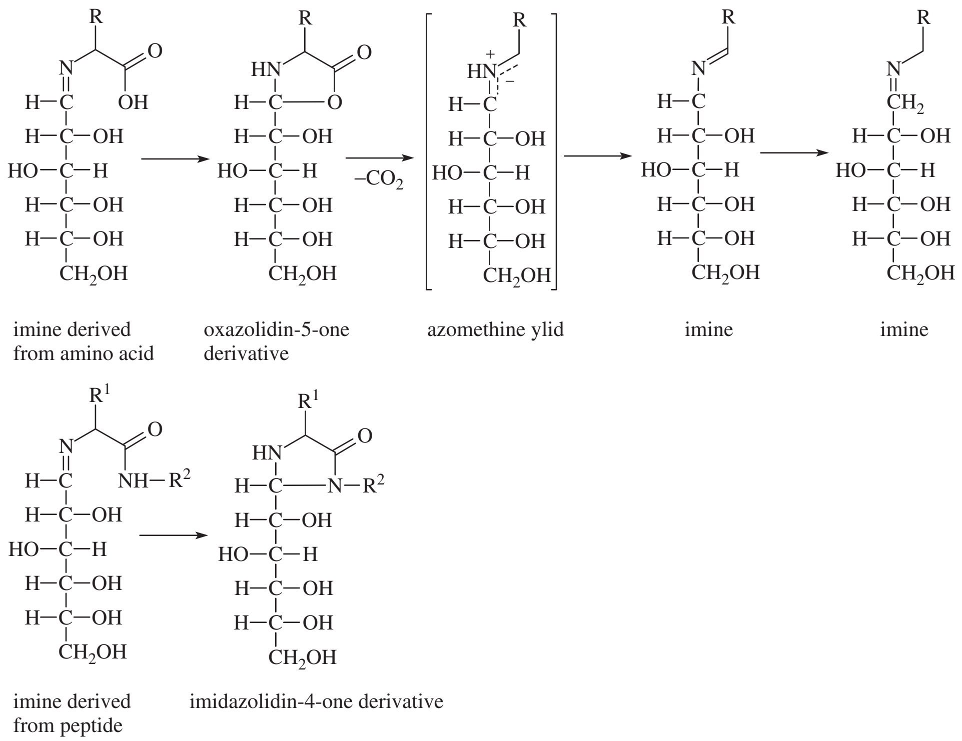


Figure 4.81 Formation of imines, imidazolidinones, oxazolidinones from imines derived from amino acids and peptides.

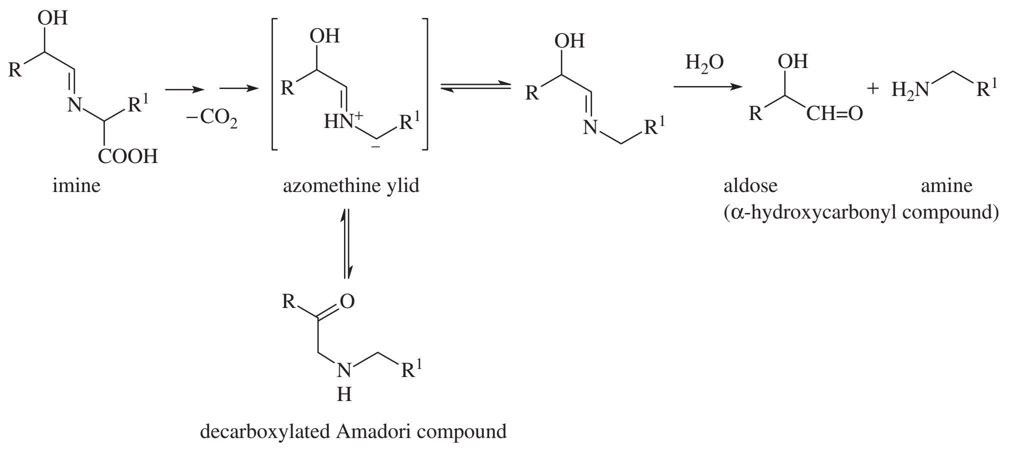


Figure 4.82 Imine degradation and formation of decarboxylated Amadori product (R = aldose or α-hydroxy compound residue, R^1 = amino acid residue).

Amadori compound (Figure 4.82), which provides the original α-hydroxycarbonyl compound after isomerisation, elimination of water and amine hydrolysis. If the decarboxylated Amadori compound is in the form of an amine, the C–N bond can be directly cleaved to form a 1-amino-2-oxo compound (1-amino-1-deoxyketose) and the original amino acid derivative with a vinyl group, as in, for example, the formation of acrylamide from asparagine. Another possibility of acrylamide formation is oxidation of glycosylated asparagine to oxoimine and its decarboxylation, which is then followed by hydrolysis to the immediate precursor of acrylamide, 3-aminopropionamide (see Section 12.2.2).

Another important reaction of *N*-glycosylamines is retroaldolisation, which generates the highly reactive C_2 intermediates glycolaldehyde *N*-alkylimines (*N*-substituted aminoacetaldehyde derivatives) and D-erythrose (Figure 4.83). Glycolaldehyde alkylimines dimerise almost immediately to *N*,*N*′-dialkyldihydropyrazines that are transformed into pyrazinium salts by two electron oxidation (Figure 4.84). Imine hydrolysis yields glycolaldehyde, while glyoxal is formed by their oxidation (Figure 4.85). Glyoxal is likewise formed by retroaldolisation of hexos-2-uloses. Reactive hydroxycarbonyl and dicarbonyl compounds (glycolaldehyde and glyoxal) can be thus formed evne in the early stage of the Maillard reaction, while the classical Hodge scheme expects the formation of these compounds in the advanced stages of the Maillard reaction by decomposition of aminodeoxysugars via 2,3-enolisation. Glyoxal, however, is also a product of lipid oxidation.

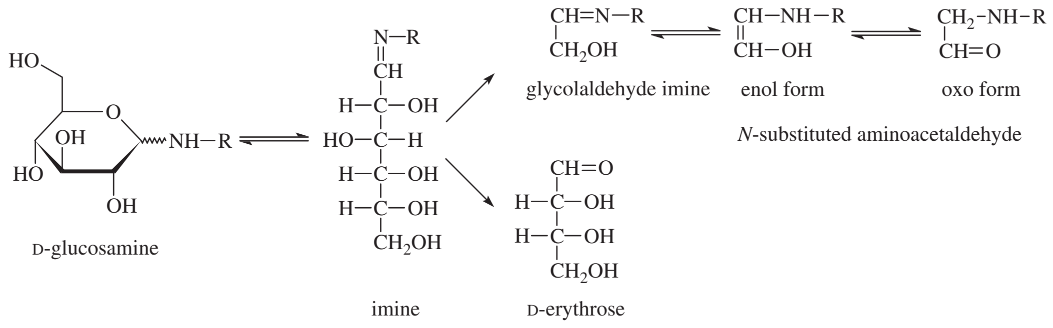


Figure 4.83 Retroaldolisation of glycosylamines.

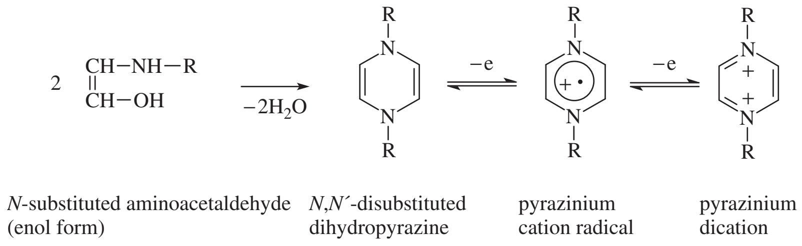


Figure 4.84 Formation of pyrazine derivatives.

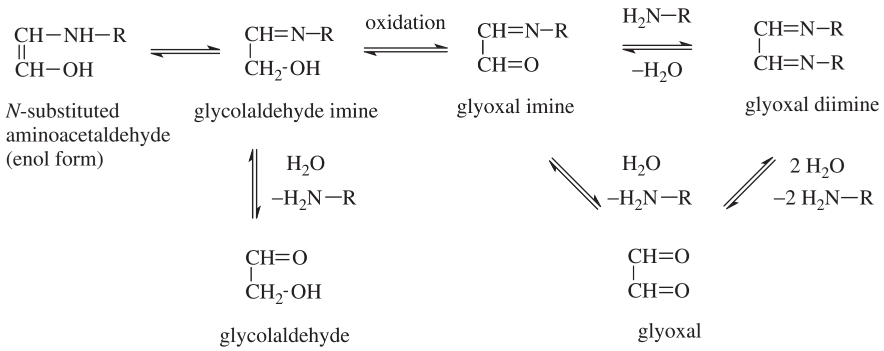
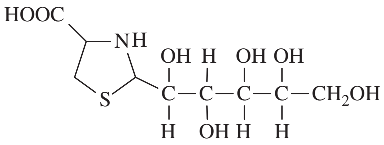
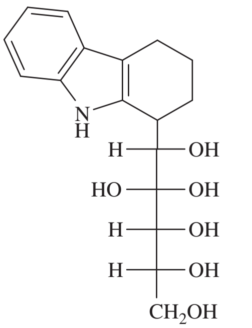


Figure 4.85 Formation of glycolaldehyde and glyoxal.

Reactions of sugars with amino acids, which have more reactive functional groups (such as sulfur, hydroxy, aromatic and heterocyclic amino acids), also bypass the Amadori rearrangement and the reaction gives rise to various heterocyclic compounds. For example, glucose yields a thiazolidine derivative, 2-(D-gluco-1,2,3,4,5-pentahydroxypentyl)thiazolidine-4-carboxylic acid (**4-213**), with cysteine and a β-carboline derivative, 1,2,3,4-tetra hydro-1-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl)-β-carboline (**4-214**), arises from tryptophan or tryptamine.



4-213, 2-(D-*gluco*-1,2,3,4,5-pentahydroxypentyl) -thiazolidine-4-carboxylic acid



4-214, 1,2,3,4-tetrahydro-1-(D-*gluco*-1,2,3,4,5 -pentahydroxypentyl)-β-carboline

4.7.5.3 Decomposition of aminodeoxysugars

All aminodeoxysugars are strong reducing agents, less reactive than reductones, but more reactive than the original sugars. Their mutarotation in aqueous solutions leads to an equilibrium mixture of different forms of aminodeoxysugars.

Diketosamines are unstable compounds that decompose to ketosamines and many other compounds, even in aqueous solutions at room temperature. Ketosamines (Amadori compounds) are relatively stable in the solid state and in neutral aqueous solutions, but their stability is significantly lower than the stability of the original sugar. In acidic and alkaline solutions, ketosamines decompose. Their degradation in alkaline solutions is faster than in acidic solutions. In addition to pH, the rate of ketosamine degradation is influenced considerably by the different stabilities of the individual cyclic forms. Their decomposition is particularly fast when ketosamines are present in the less stable furanose or acyclic form. For example, ketosamines formed in the reaction of glucose with amino acids (derivatives of 1-amino-1-deoxyfructose) at 20 °C are present in the form of β-pyranose (approximately 64%), α-furanose (15%), β-furanose (15%) and α-pyranose (6%). The equilibrium mixture contains the acyclic form in quantities up to 2%. The composition of the equilibrium mixture varies only slightly with pH. Decomposition of aldosamines proceeds in a similar way to the decomposition of ketosamines, with the difference that some original ketose also arises.

Decomposition of ketosamines starts, like most other reactions of monosaccharides, by 1,2-enolisation that yields an amino analogue of 1-ene-1,2-diol, which is 1-ene-1-amino-2-ol (Figure 4.86). The reaction proceeds by elimination of the C-3 hydroxyl group and hydrolysis of the bound amino compound provides 3-deoxy-D-*erythro*-hexos-2-ulose and, further, 3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose, 5-hydroxymethylfuran-2-carbaldehyde in addition to other products that are formed by degradation of glucose by 1,2-enolisation.

2,3-Enolisation of ketosamines (Figure 4.86) gives 2-ene-2,3-diol as a primary intermediate and decomposition of the bound amino compound yields 1-deoxy-D-*erythro*-hexo-2,3-diulose as the main intermediate. The same compound is formed to a small extent from fructose in the absence of amino compounds. A minor reaction product is 4-deoxy-D-*glycero*-hexo-2,3-diulose. Both uloses are transformed into a variety of products by subsequent reactions, similar to in the absence of amino compounds, but in higher yields.

It is obvious that the decomposition of aminodeoxysugars by 1,2-enolisation and 2,3-enolisation gives rise to the same key intermediates that also arise during the degradation of sugars in the absence of amino compounds (3-deoxy-D-*erythro*-hexos-2-ulose, 1-deoxy-D-*erythro*-hexo-2,3-diulose and 4-deoxy-D-*glycero*-hexo-2,3-diulose). The fundamental difference is that these and many other products are produced from carbohydrates in the absence of

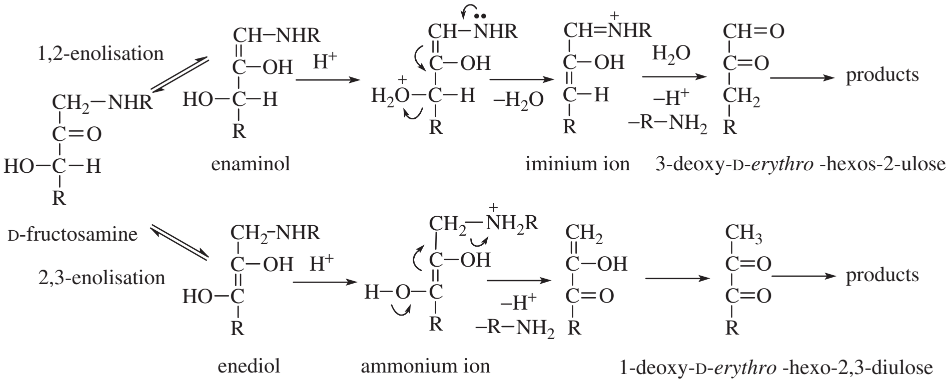


Figure 4.86 1,2-Enolisation and 2,3-enolisation of ketosamines.

amine only in strongly acidic or strongly alkaline media (pH lower than 3 or higher than 8) or at higher temperatures (e.g. during caramelisation). Amino compounds catalyse the degradation of saccharides, therefore the reaction rate is higher and degradation of aminodeoxysugars proceeds under normal temperatures and in virtually a neutral media, which are typical for most foods (pH 4–7). Reactions differ too in terms of the quantitative representation of individual products. In the absence of amino compounds, mainly 3- and 4-deoxyuloses are formed, while in the presence of amino compounds the characteristic products are 1-deoxyuloses. A number of degradation products of sugars, including deoxy sugars, furans, pyrans and fragments containing reactive carbonyl groups, also react with amino compounds with the formation of compounds containing nitrogen or sulfur in the molecule in the case where sulfur amino acids and other sulfur compounds are involved these reactions. The characteristic products of the Maillard reaction are, therefore, nitrogen and sulfur-containing heterocyclic compounds.

Factors that determine whether aminodeoxysugars undergo 1,2-enolisation or 2,3-enolisation have been studied for decades, but the reaction mechanisms are still not fully understood. It is generally considered that 1,2-enolisation takes place in acidic media where the nitrogen atom of the aminodeoxysugars is protonated. The level of 1,2-enolisation products, such as derivatives of furan-2-carbaldehyde, also increases with increasing amino compound basicity. Aminodeoxysugar must be present as the free base in order to allow the 2,3-enolisation. For this reason, 2,3-enolisation occurs especially in alkaline or non-aqueous media, for example during caramelisation.

According to recent surveys, ketosamines degrade preferentially via 1,2-enolisation and via 2,3-enolisation under milder reaction

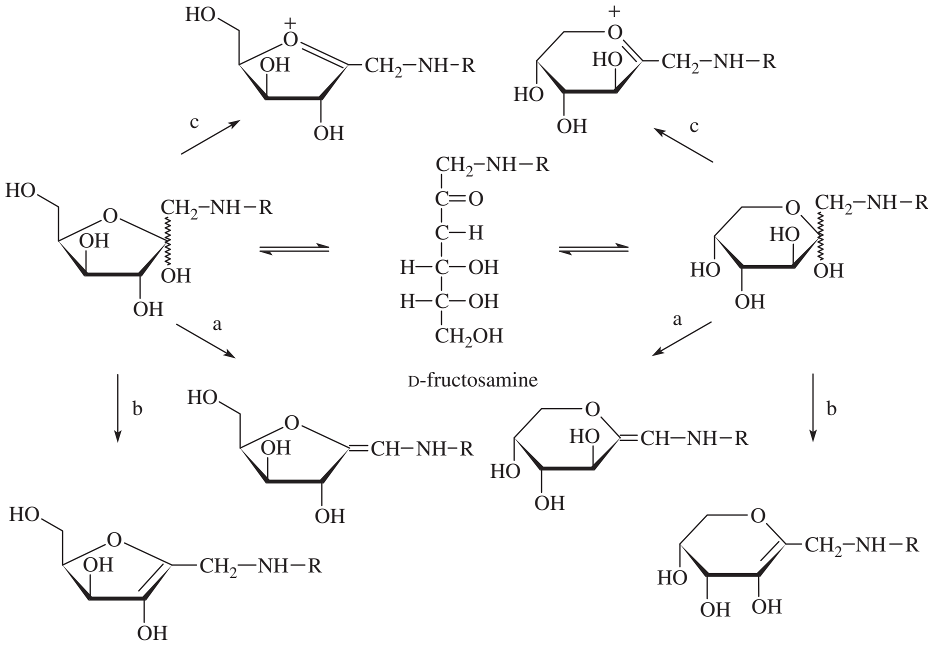


Figure 4.87 Ketosamine degradation by 1,2-dehydration (a), 2,3-dehydration (b) and 2-dehydroxylation (c).

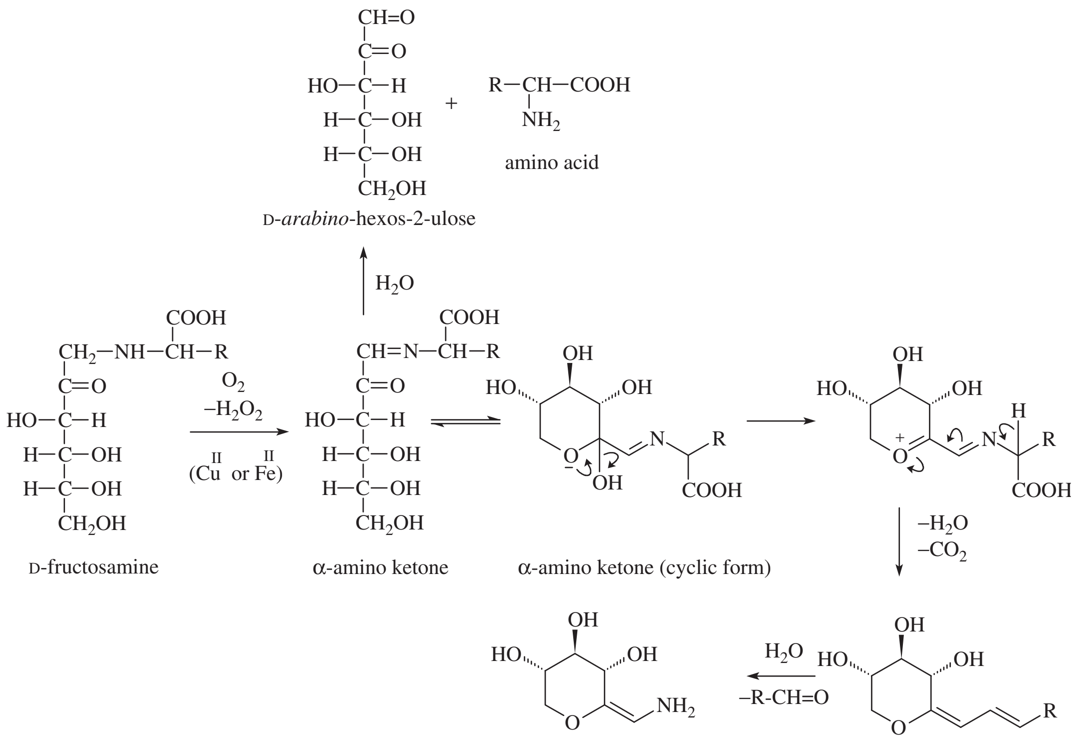


Figure 4.88 Oxidation of ketosamines to D-*arabino*-hexos-2-ulose and Strecker aldehydes.

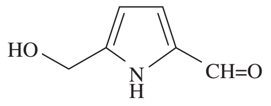
conditions (in solutions at relatively low temperatures) as mentioned previoulsy. At higher temperatures, however, ketosamine decomposition prevails by 1,2-dehydration, 2,3-dehydration and the so called *O*,2-dehydroxylation, which is an elimination of the hemiacetal hydroxyl group (Figure 4.87). It is assumed that the 1,2-dehydration occurs primarily in furanoses, while the 2,3-dehydration mechanisms predominates in pyranoses. The 2,3-dehydration products of pyranoses are stabilised by hydrogen bonds between nitrogen atoms and C-3 hydroxyl groups.

Another important reaction is decomposition of amino acid derived ketosamines in the presence of oxygen, which is catalysed by transition metal ions. Products of this decomposition are glycos-2-uloses. For example, D-fructosamine yields D-*arabino*-hexos-2-ulose and in addition to other products, this reaction produces the corresponding Strecker aldehyde (Figure 4.88).

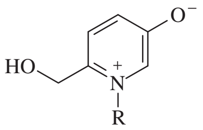
4.7.5.3.1 Decomposition of 3-deoxyglycosuloses

The main products of 3-deoxy-D-*erythro*-2-hexos-2-ulose degradation are (*Z*)-3,4-dideoxy-D-*glycero*-hex-3-enos-2-ulose (2-hydroxy-6-hydroxymethylpyran-3-one), the corresponding (*E*)-isomer and 5-hydroxymethylfuran-2-carbaldehyde.

Except for furan derivatives in the case of 3-deoxyglycos-2-ulose decomposition, considerable amounts of pyrrole derivatives, *N*-substituted 5-hydroxymethylpyrrole-2-carbaldehydes (**4-215**) and their isomeric pyridines (pyridinium betaines, **4-216**) are formed in the presence of excess amine. These compounds are formed in only trace amounts in the reaction of 5-hydroxymethylfuran-2-carbaldehyde with amino compounds, which suggests that incorporation of an amino compound occurs preferentially in a reaction with its precursors. If the reacting amino compound is ammonia, nitrogen atoms of the resulting pyrrole and pyridine derivatives are unsubstituted. In the presence of amino acids, the reaction products are carboxymethyl substituted 1-pyrrole-2-carbaldehydes (**4-215**). Their cyclisation yields the corresponding lactones (**4-217**). Lactams (dihydropyrrolopyrazinones, **4-218**) are formed in the presence of peptides.



4-215, 5-hydroxymethylpyrrol-2-carboxaldehyde



4-216, pyridinium betaines

The C-1/C-2 bond cleavage of 3-deoxy-D-*erythro*-hexos-2-ulose yields formic acid and 2-deoxy-D-*erythro*-pentose, which is transformed into 2-furfuryl alcohol and, in the presence of

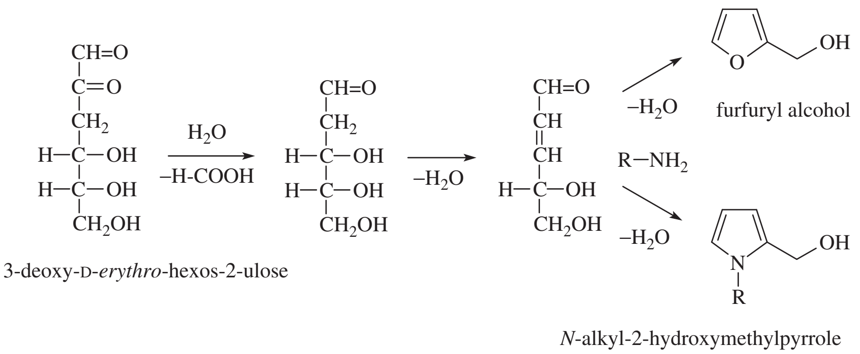
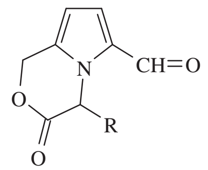
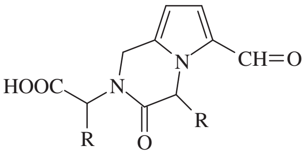


Figure 4.89 Degradation of 3-deoxy-D-*erythro*-hexos-2-ulose.



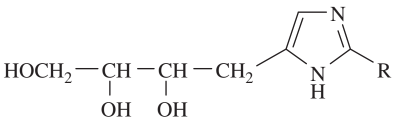
4-217, lactones of *N*-carboxymethyl-5-hydroxymethylpyrrole-2-carboxaldehydes



4-218, dihydropyrrolopyrazinones

amino compounds, into *N*-substituted 2-hydroxymethylpyrroles (Figure 4.89). These substances tend to form polymers. Pyrrole and pyridine derivatives also form in reactions of amino compounds with 3-deoxyglycos-2-uloses derived from disaccharides. Another mechanism for the formation of 2-furfuryl alcohol from glucose in aqueous systems involves the oxidation of glucose to gluconic acid, which is decarboxylated to a pentitol and then followed by dehydration and cyclisation. In wort, *Saccharomyces cerevisiae* yeast can reduce furan-2-carbaldehyde to 2-furfuryl alcohol, but the main pathway during boiling of the wort seems to be the decomposition of the Amadori product derived from maltose via 3-deoxy-D-ribulose, which dehydrates to 2-furfuryl alcohol.

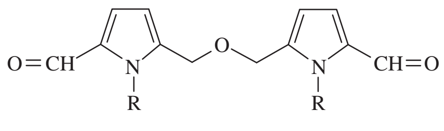
Reactions of 3-deoxyhexos-2-uloses with ketosamines derived from ammonia yield polyhydroxyalkyl substituted pyrroles and reactions of ketosamines derived from amines produce *N*-substituted polyhydroxyalkyl pyrroles. When the aforementioned uloses react with ketosamines in the presence of ammonia, 2,5-polyhydroxyalkyl substituted pyrazines are formed (Figure 4.90). These compounds also arise by dimerisation of ketosamines. 2,6-Disubstituted analogues of these compounds are formed by condensation of aldosamines with ketosamines. Polyhydroxyalkyl substituted imidazoles (**4-219**) are formed as additional products in reactions of 3-deoxyglycos-2-uloses with ammonia. It is assumed that all polyhydroxyalkyl substituted heterocyclic compounds can be transformed into normal alkyl substituted analogues (such as dimethyl derivatives) during thermal operations.



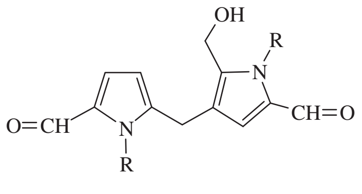
4-219, 1,4-polyhydroxyalkyl substituted imidazoles

3-Deoxyglycos-2-uloses react differently with secondary amines than with primary amines. Usually significant amounts of cyclic compounds form, but 5-hydroxymethylfuran-2-carbaldehyde does not arise at all (or only in small amounts). For example, the reaction of proline with 3-deoxy-D-*erythro*-hexos-2-ulose yields the so-called maltoxazine, a flavour-active compound occurring in beer and malt (Figure 4.91).

Condensation of *N*-substituted pyrrole-2-carbaldehyde derivatives mainly leads to coloured products, examples of which are compounds **4-220** and **4-221**. Derivatives of furan-2-carbaldehyde and many other furans are also significantly involved in non-enzymatic browning reactions. Reactions of furan-2-carbaldehyde with amino acids produce common Maillard reaction products, such as imines. Subsequent reactions of imines produce more complicated structures, mostly coloured compounds (Figure 4.92). The product illustrated in **4-222** forms



4-220, ethers of *N*-substituted 5-hydroxymethylpyrrole-2-carboxaldehydes



4-221, *N*-substituted 5-hydroxymethylpyrrol-2-carboxaldehyde dimers

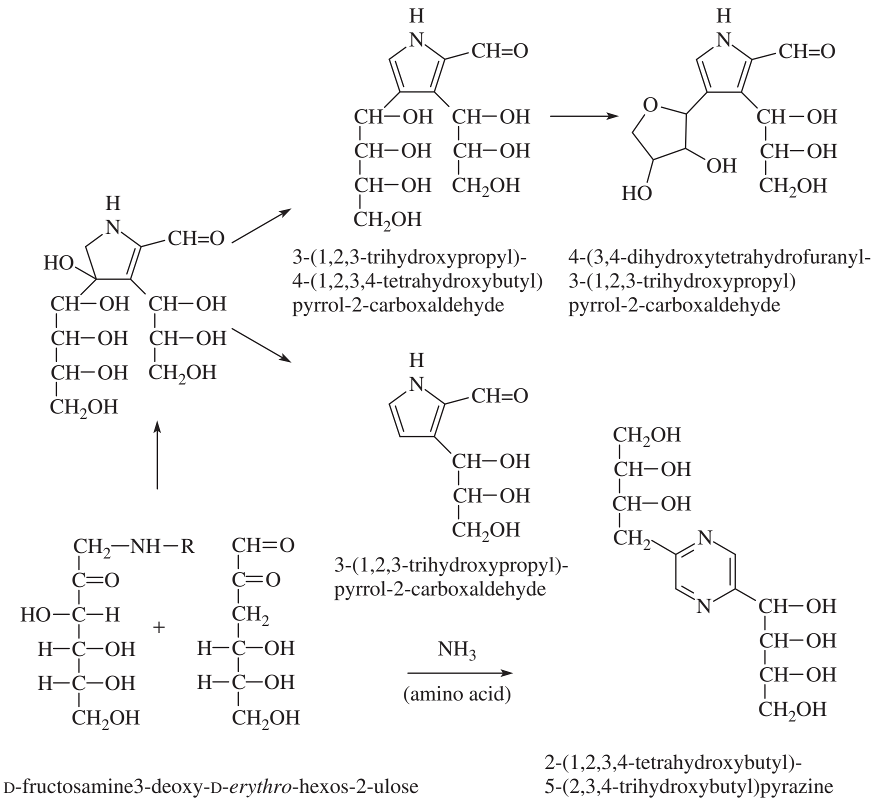


Figure 4.90 Formation of polyhydroxyalkyl substituted pyrrole-2-carbaldehyde and pyrazine derivatives (R = H).

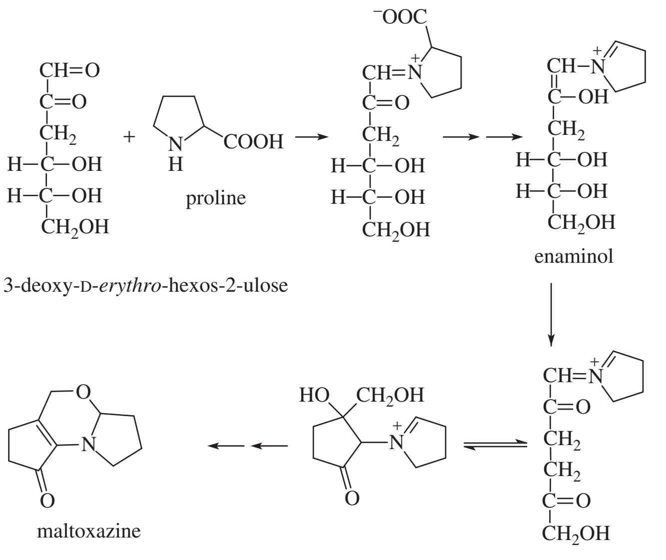


Figure 4.91 Formation of maltoxazine.

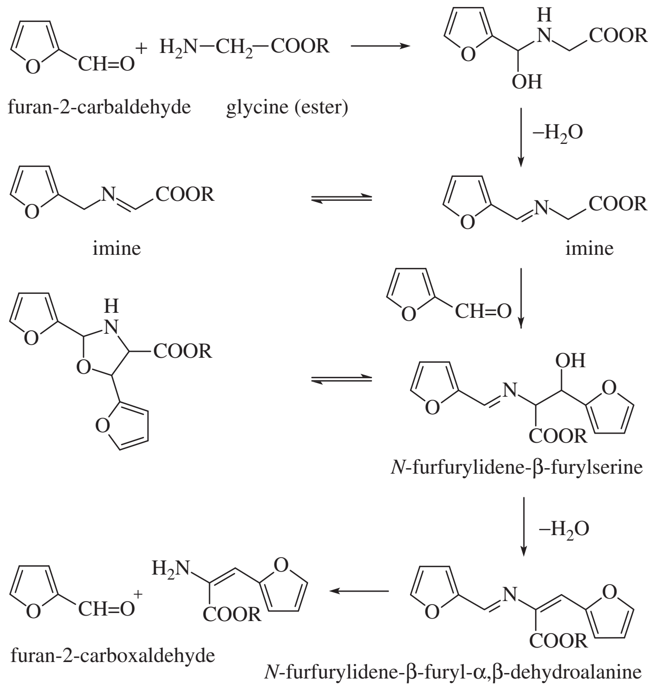
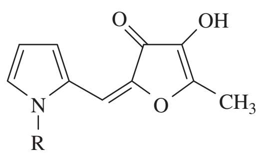


Figure 4.92 Reaction of furan-2-carbaldehyde with glycine.



4-222, reaction product of primary amines with 4-hydroxy-5-methyl-2*H*-furan-3-one

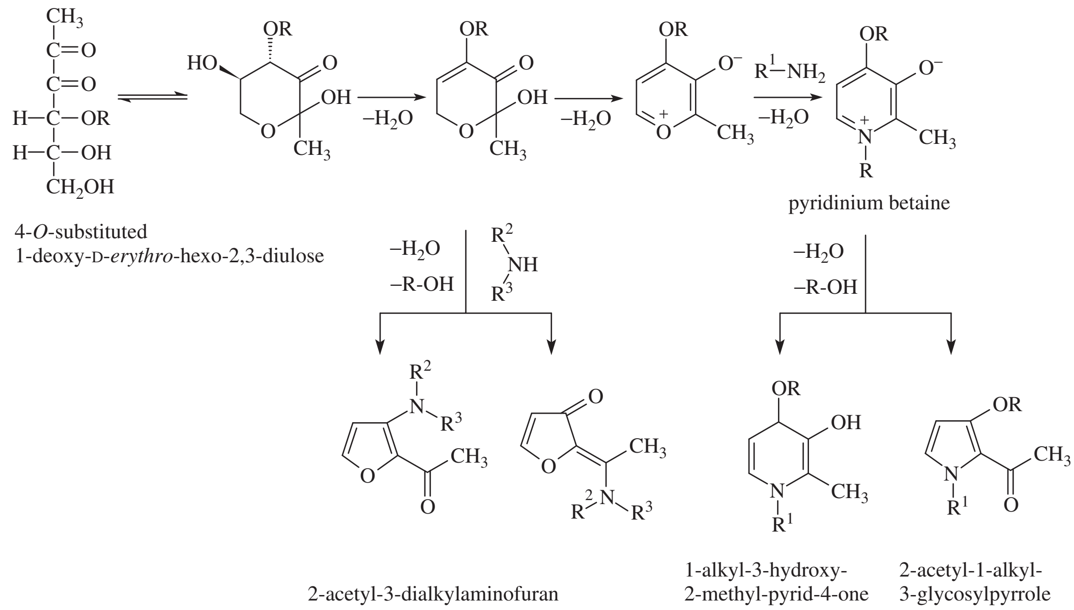
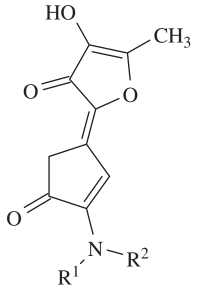


Figure 4.93 Reaction of primary and secondary amines with 1-deoxyglyco-2,3-diuloses derived from disaccharides.

in the presence of primary amines. The reaction of 4-hydroxy-5-methyl-2*H*-furan-3-one with secondary amines yields the major coloured product shown in **4-223**.

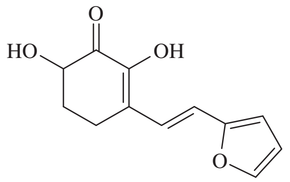


4-223, reaction product of secondary amines with 4-hydroxy-5-methyl-2*H*-furan-3-one

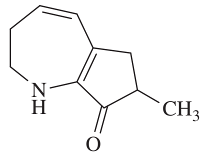
4.7.5.3.2 Decomposition of 1-deoxyglycodiuloses

1-Deoxyglyco-2,3-diuloses are highly reactive compounds that scientists have so far failed to isolate from either foods or model reaction systems. Their presence has been indirectly confirmed by analysis of suitable derivatives. Important degradation products of 1-deoxyhexo-2,3-diuloses are furanones.

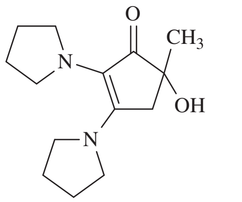
In the presence of sulfur compounds (e.g. cysteine) pentoses and methylpentoses produce, in addition to 4-hydroxy-5-methyl-2*H*-furan-3-one and 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one, respectively, related compounds containing sulfur in the molecule. Many of these compounds resemble the aroma of cooked or roasted meat. In the presence of primary amines, 1-deoxyhexo-2,3-diuloses derived from disaccharides yield pyridinium betaines and, further, pyrid-4-ones and isomeric 2-acetylpyrroles. The reaction with secondary amines leads to *N*-substituted furan derivatives (Figure 4.93). 1-Deoxyhexo-2,3-diuloses also condense with carbonyl compounds and form coloured products (**4-224**). The reaction with proline or hydroxyproline results in cyclopenta[*b*]azepin-8(1*H*)-ones (**4-225**) and bispyrrolidinohexose reductones (**4-226**).



4-224, reaction products of 1-deoxyhexo-2,3-diuloses with furan-2-carboxaldehyde



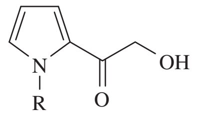
4-225, reaction product of 1-deoxyhexo-2,3-diulose with proline



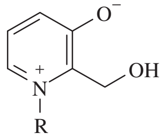
4-226, reaction product of 1-deoxyhexo-2,3-diulose with two molecules of proline

4.7.5.3.3 Decomposition of 4-deoxyglycosuloses and 1-amino-1,4-dideoxyglycosuloses

In the presence of amino compounds, the decomposition of 4-deoxyglycosuloses, such as 4-deoxy-D-*glycero*-hexo-2,3-diuloses (whose existence is expected during the transformation of lactulose and fructose into 2-hydroxyacetylfuran) yields the corresponding pyrroles (**4-227**) and isomeric pyridinium betaines (**4-228**). These pyridinium betaines, however, differ from pyridinium betaines (**4-216**) formed by decomposition of 3-deoxyglycos-2-uloses.



4-227, 2-hydroxyacetylpyrroles

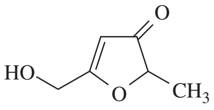


4-228, pyridinium betaines

In the Maillard reaction of oligosaccharides with a 1,4-glycosidic bond (such as maltose, lactose and lactulose) in media of low water activity, the 1-deoxyhexo-2,3-diuloses, 3-deoxyhexos-2-uloses and short-chain α-dicarbonyl compounds that are formed represent only a minor portion of the dicarbonyl intermediates. An important intermediate is the amino analogue of 4-deoxy-D-*glycero*-hexo-2,3-diulose, that is, 1-amino-1,4-dideoxy-D-*glycero*-hexo-2,3-diulose. Probably the most common product of decomposition of this diulose is 2-aminoacetylfuran, which arises (analogously to 2-hydroxyacetylfuran) by cyclisation and dehydration. Aminoacetylfuran is stable in acidic media, but at pH higher than 5 readily oxidises and condenses to form more complex structures (Figure 4.94). In cases where the amino compound bound in aminoacetylfuran is protein (bound by the ε-amino group of lysine), the resulting aminopyrrole forms cross-links, which connect the peptide chains of the proteins. Aminoacetylfurans react with ammonia with the formation of imidazole derivatives.

The main dicarbonyl product of the Maillard reaction with oligosaccharides that have 1,4-glycosidic bonds are 1,4-dideoxyhexo-2,3-diuloses (Figure 4.95). 2,3-Enolisation of the Amadori compound is followed by splitting of the oligosaccharide residue by β-elimination to form 1-amino-1,4-dideoxy-D-*glycero*-hexo-2,3-diulose. Reduction and hydrolysis of the bound amino acid yields 1,4-dideoxy-D-*glycero*-hexo-2,3-diulose. The residue of the reducing oligosaccharide with a 1,4-glycosidic linkage in the reducing end can be transformed in this way repeatedly. 1,4-Dideoxyhexo-2,3-diuloses may also result from 1-deoxyhexo-2,3-diuloses, which are reduced during the Strecker degradation of amino acids.

1,4-Dideoxyhexo-2,3-diuloses are unstable compounds and their cyclisation and dehydration leads, for example, to 5-hydroxymethyl-2-methylfuran-3-one (**4-229**). Cyclisation and dehydration of 1-amino-1,4-dideoxyhexosulose produce aminoacetylfurans. The most famous of these products is furosin. In non-acid media, maltooligosaccharides and other (1→4)-oligosaccharides give rise, via 1-amino-1,4-dideoxyhexosuloses, to α-dicarbonyl compounds with five carbon atoms, namely 3-deoxy-D-*glycero*-pentulose, 3-deoxy-D-*glycero*-pentos-2-ulose and 3,4-dideoxypentosulose (Figure 4.32).



4-229, 2-methyl-5-hydroxymethyl-2*H*-furan-3-one

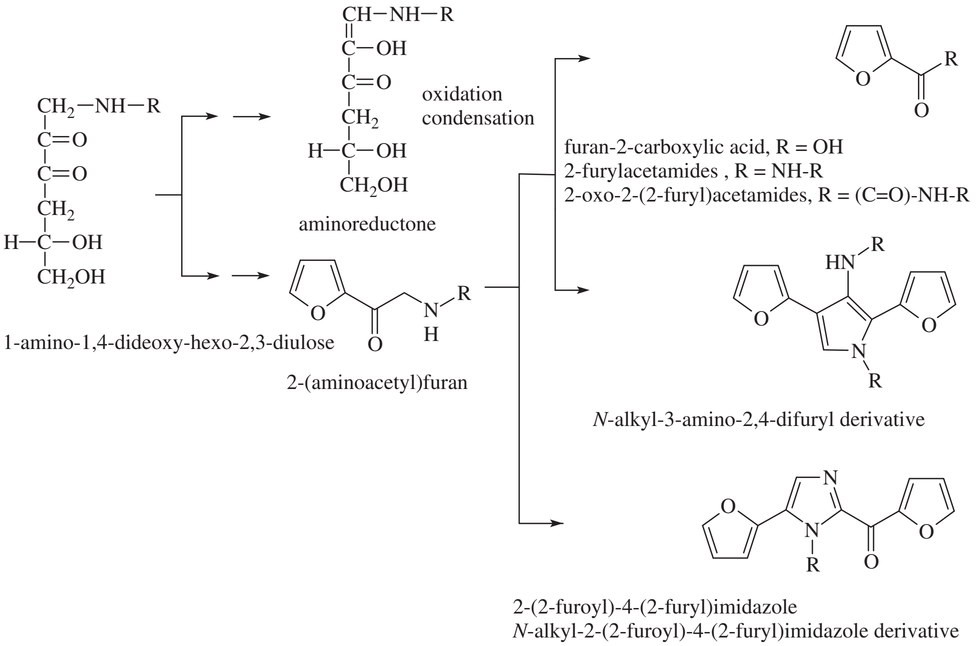


Figure 4.94 Important reactions of 1-amino-1,4-dideoxyhexo-2,3-diuloses.

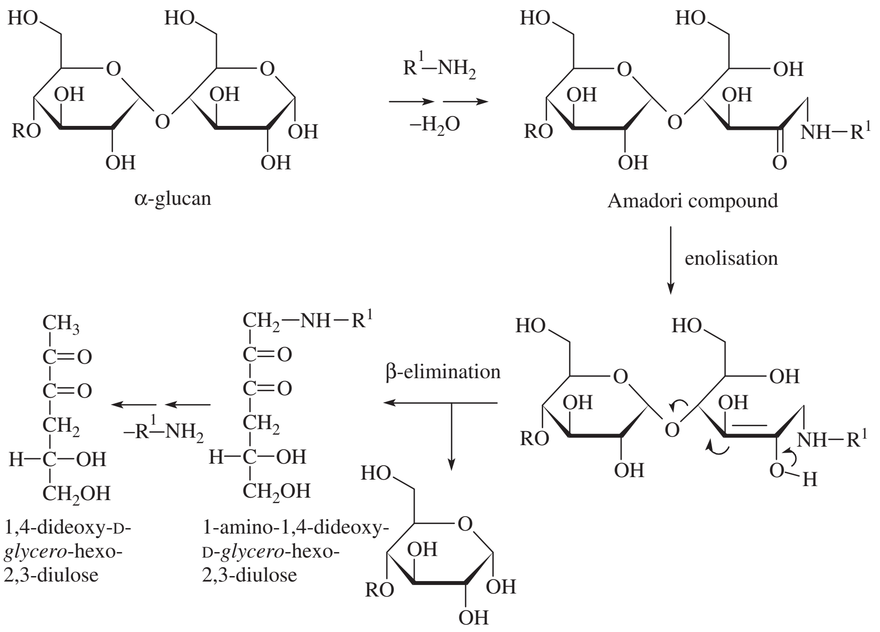
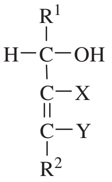


Figure 4.95 Formation of diuloses from maltooligosaccharides.

The key intermediates in the formation of furan and pyrrole derivatives from 3-deoxyglycos-2-uloses via β-dicarbonyl compounds are 3-deoxy-2,4-diuloses and 1-amino-1,3-dideoxy-2,4-diuloses. Cyclisation, dehydration and keto-enol tautomerisation of diuloses yield 2-acetylpyrrole derivatives as the main products (Figure 4.96).

4.7.5.4 Formation of aminoreductones

1-Deoxyhexo-2,3-diuloses can be easily converted into aliphatic aminoreductones of the general structure given by formula **4-230**. Aliphatic aminoreductone derived from propylamine was the first linear six carbon chain aminoreductone, whose formation in the Maillard reaction was assumed and then was later identified (Figure 4.94). A very reactive compound, formed by degradation of 1-deoxyhexodiuloses, is diacetylformoin (Figure 4.40, R = H). This compound, existing in several tautomeric forms, reacts with primary amines to form pyrrolid-3-one derivatives that are transformed into methylene reductic acid (R = H) (Figure 4.97). Reductic acid forms analogously from pentoses and from ascorbic acid. Diacetylformoin reacts with secondary amines to form 5-dialkylamino substituted 2*H*-dihydrofuran-3-one derivatives. The reaction of diacetylformoin with the ε-amino group of lysine bound in proteins causes protein cross-linking. Aminohexose reductones and derivatives of pyrrolidone derived from disaccharides (R = glycosyl, Figure 4.97) are formed by similar reactions.



4-230, aminoreductones

X = OH, NR_2 or NHR
Y = OH, NR_2 or NHR

4.7.5.5 Strecker degradation of amino acids

The mechanism of this very important reaction is given elsewhere (Figure 2.43). In addition to numerous other substances, the active compounds are also aldoses, ketoses, derivatives of furan-2-carbaldehyde, α-dicarbonyl compounds derived from sugars, such as glycos-2-uloses or glyco-2,3-diuloses, and in particular the simple decomposition products of sugars, such as glyoxal and methylglyoxal.

The importance of Strecker degradation is mainly in the formation of reactive and often sensory active aldehydes and ammonia (free or bound in reactive α-amino carbonyl compounds). Strecker aldehydes, together with α-amino carbonyl compounds, contribute significantly to the formation of many heterocyclic compounds (which are important flavour-active components of processed foods) and the brown pigments melanoidins. Aldoses simultaneously produce the corresponding 2-deoxyaldoses or 1-amino-1-deoxyalditols and 2-oxoacids.

4.7.5.6 Melanoproteins

During processing and storage of foods, numerous reactions lead to protein structure modification. As well as denaturation and addition reactions, which lead to the formation of disulfide bridges and reversible oxidation of side chains of bound amino acids, isopeptide

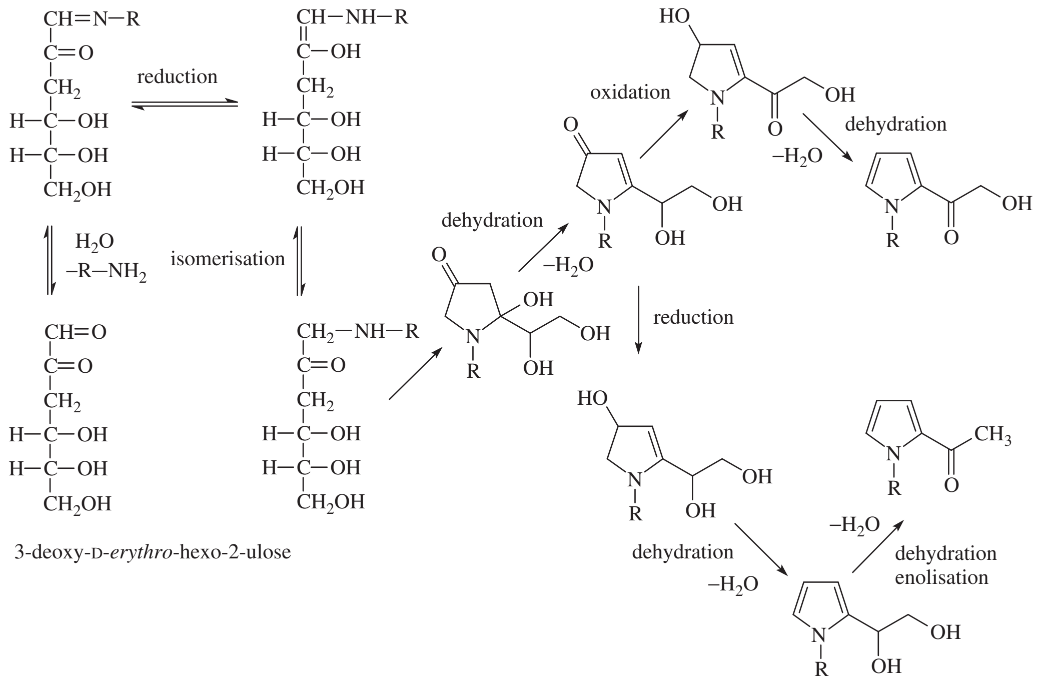


Figure 4.96 Transformation of 3-deoxy-D-*erythro*-hexos-2-ulose in the presence of amino compounds.

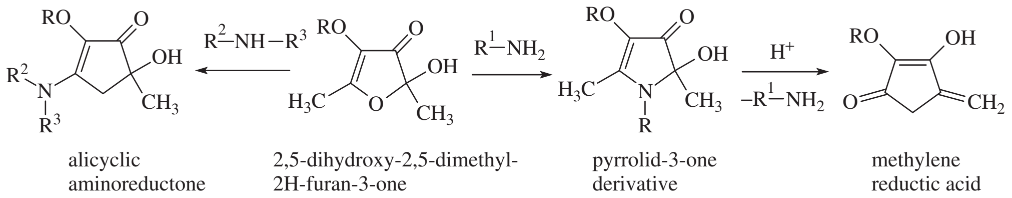


Figure 4.97 Reaction of diacetylformoin with amines.

bonds (Figure 2.50) or to the formation of amino acids derived from dehydroalanine (Figure 2.53), modifications of protein molecules are also caused by reactive dicarbonyl compounds formed during transformations of reducing sugars or products formed during oxidation of lipids. These bifunctional carbonyl compounds form adducts with one or two reactive amino acid residues in the peptide chains, which leads to inter- and intramolecular cross-linking of proteins. These reactions are of especially great importance for functional properties of proteins and likewise adversely affect their nutritional value. Proteins modified by reactions with carbonyl intermediates of the Maillard reaction or with secondary lipid oxidation products are often referred to as **melanoproteins**.

Significant in terms of health risks are spontaneous non-enzymatic reactions in living organisms that lead to the same or similar modifications of native protein as during food processing. This so-called protein **glycation** (or glycosylation) plays an important role both in physiological and pathological processes in the organisms. The modified protein, or strictly the modified amino acids released from the protein, are known by the acronym AGE (advanced glycation end products). Analogous structures abbreviated ALE (advanced lipoxidation end products) are produced by reactions of proteins with certain products of lipid oxidation (see Section 3.8.1.12.1). Excessive reactions are an indicator of tissue aging and pathological manifestations of certain diseases, such as increased oxidative stress due to hyperglycaemia in patients with type 2 diabetes. AGEs accumulate at sites of microvascular injury in diabetes, including the kidney, the retina and within the vasculature. One of the causes of oxidative stress in aging, diabetes and other diseases is considered to be the formation of oxygen radicals from glycated proteins. For example, even the Amadori compounds (products of early glycation of proteins) can apparently generate the superoxide radical anion, which arises from hydrogen peroxide in the presence of oxygen. It can react with traces of metal ions to form hydroxyl radicals. The potential adverse effects on health of diet-derived AGEs is of current interest, due to their proposed involvement in the disease progression of diabetic and uraemic conditions.

The enhanced formation of AGEs also exists in various other diseases, such as atherosclerosis, Alzheimer's disease, end-stage renal disease (ESRD), rheumatoid arthritis and liver cirrhosis. Whether AGEs are also risk factors for these diseases is still unclear. Most modifications *in vivo* are found in proteins with long life, such as collagens, myelin or α-crystalline. For example, a gradual change in the structure of collagen during aging causes a decrease

in the tissue elasticity. Ultimately, these changes lead to restrictions of joint mobility, decreased muscle performance, changes in the cardiovascular system or changes in the ocular lens. Modification of proteins may similarly have a negative impact in cases where AGEs are located in areas of interaction with other protein chains, the substrate in interaction in the enzyme–substrate or DNA during transcription.

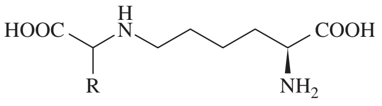
AGEs can arise not only from glucose, but also from the reactive products of glucose metabolism and non-enzymatic degradation. The most important Maillard reaction products reacting with proteins are methylglyoxal, glyoxal and 3-deoxyhexosuloses, but also identified are other precursors of AGEs, namely α-hydroxycarbonyl products of sugar fragmentation, transformation products of ascorbic acid or some secondary decomposition products of lipid hydroperoxides. Some common intermediates of sugar metabolism also contribute to the formation of AGEs *in vivo*. For example, triose phosphates or fructose phosphates are effective modifiers of proteins.

The most frequently modified part of the protein is the ε-amino group of protein-bound lysine and the guanidyl group of protein-bound arginine, which are involved in the cross-linked AGEs formation. They react with bifunctional reagents (such as dicarbonyl compounds) and the product then reacts through the other functional group with another lysine or arginine residue of the same or another protein molecule. Tryptophan and histidine bound in proteins react with only one functional group of bifunctional reagents yielding non-cross-linked AGEs. Little is known about the reactions of bound cysteine, whose thiol group is a better nucleophile than the amino groups of lysine or arginine. Cysteine conjugates can significantly influence the technological and physiological properties of glycated proteins and also serve as a carrier and reservoir of reactive dicarbonyl compounds.

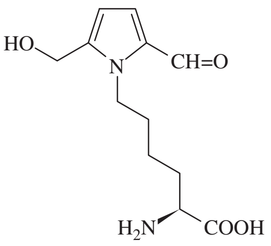
AGEs are released by controlled physiological or enzymatic hydrolysis of modified proteins as modified low molecular weight peptides or modified amino acids. The number of AGEs identified up-to-now may be just the tip of the iceberg, because different types of modifications are formed in tissue proteins and the products that usually occur only in trace quantities are difficult to identify. Adducts identified after hydrolysis are called by trivial names and acronyms. They can be classified according to the structure, the participating amino acids or carbonyl compounds, the origin of the precursors (carbohydrates, their degradation products, oxidation products of lipids or both groups of these nutrients) or by whether or not the AGEs are cross-linked or not. Many cross-linked products are fluorescent, while some of them are coloured or have reducing properties.

The non-cross-linked AGE N^{ε}-(carboxymethyl)lysine (CML) was the first stable AGE to be discovered. CML is present in a range of heat treated foods and a major AGEs structure is formed *in vivo* by oxidative cleavage of Amadori products between C-2 and C-3 of the carbohydrate chain (glyoxal) or by a gradual degradation of lactulosyllysine in dairy products (see Figure 4.102 later). It is a marker of oxidative stress and long-term damage to protein in clinical studies (aging, atherosclerosis and diabetes). Protein modification has been demonstrated in collagen, eye lenses, serum proteins or erythrocytes, among others. Together with some other AGEs, CML has also been demonstrated in lipofuscin granules, the intracellular proteolipid pigment that causes of age related spots. CML is also a marker of the Maillard reaction in foods. For example, cereal (26 mg/kg food) and fruit and vegetable (1.3 mg/kg food) categories have the highest and lowest level of CML.

The CML homologue N^{ε}-(carboxyethyl)lysine (CEL, **4-231**) forms in analogous intracellular reactions with methylglyoxal and is an important marker for age-dependent disease, such as cardiovascular disease in diabetic patients. The reaction of 3-deoxyhexosuloses with the ε-amino group of bound lysine yields pyrraline, bound 6-(2-formyl-5-hydroxymethylpyrrol-1-yl)-L-norleucine (**4-232**), identified in hydrolysates of milk proteins and found in higher levels in serum of patients with diabetes, arteriosclerosis and Alzheimer's disease (Figure 4.98).



4-231, CML, R = H
CEL, R = CH_3



4-232, pyrraline

The reaction of two lysyl residues with two molecules of glucose creates the so-called crossline (cross-linked AGE) (**4-233**), which is found in the serum and kidneys of diabetic patients. An analogous structure, called fluorolink (**4-234**), actually arises by dehydration and oxidation of crossline. Another cross-linked AGE is imidazolium salt of glyoxal-lysine dimer, trivially called GOLD (**4-235**), which is 2-ammonio-6-[1-(5-ammonio-6-oxido-6-oxohexyl) imidazolium-3-yl]hexanoate. The compound known as MOLD, (2-ammonio-6-[1-(5-ammonio-6-oxido-6-oxohexyl)-4-methylimidazolium-3-yl] hexanoate (**4-236**), results from the analogous reaction with methylglyoxal. These examples, however,

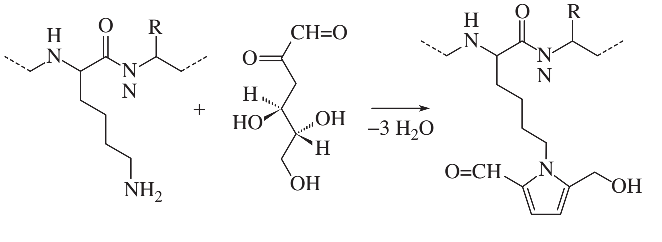
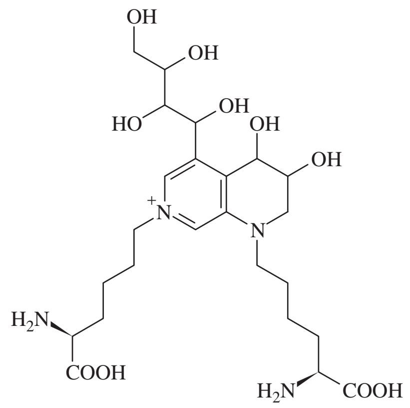
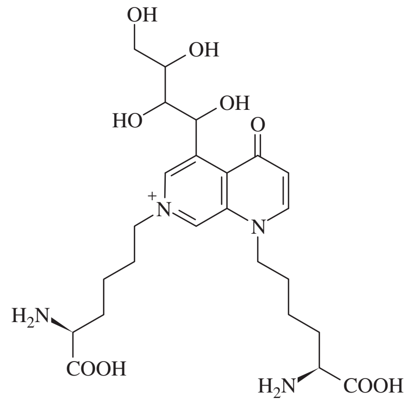


Figure 4.98 Reaction of 3-deoxyhexos-2-uloses with bound lysine to form pyrralines.

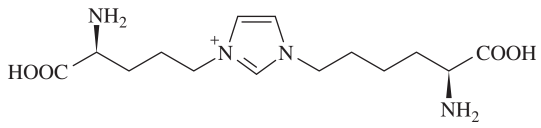
do not cover all known AGEs that are involved in cross-linking of two lysyl residues. The non-cross-linked AGE referred to as argpyrimidine, N^{δ}-(5-hydroxy-4,6-dimethylpyrimidin-2-yl)-L-ornithine (**4-237**), is derived from the reaction of methylglyoxal with the arginine protein residue. Reactions of methylglyoxal (MG) (**4-238**) or 3-deoxyglycosuloses (DG) (**4-239**) with an arginine residue yield the corresponding imidazolones, MG-imidazolone and 3DG-imidazolone. Analogous imidazolones are formed in reactions of methylglyoxal and 3-deoxyglycosuloses with protein-bound lysine. 3,4-Dideoxypentosulose formed exclusively from oligosaccharides with a (1→4) glycosidic bond is another dicarbonyl precursor in the modification of arginine residues and formation of (N^{δ}-[5-(3′-hydroxypropyl)-4-oxoimidazolon-2-yl]-L-ornithine, known as PIO (**4-240**), in foods containing reducing (1→4)-disaccharides, such as lactose, maltose or lactulose.



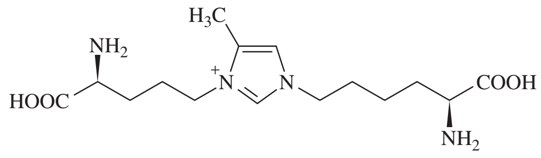
4-233, crossline



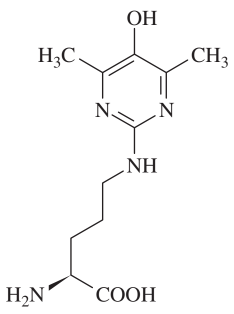
4-234, fluorolink



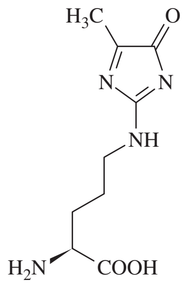
4-235, GOLD



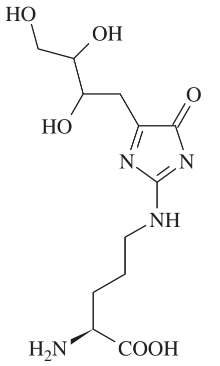
4-236, MOLD



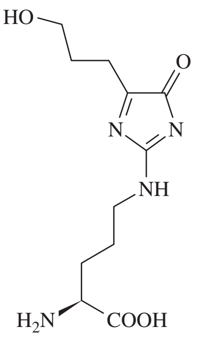
4-237, argpyrimidine



4-238, MG-imidazolone derived from arginine

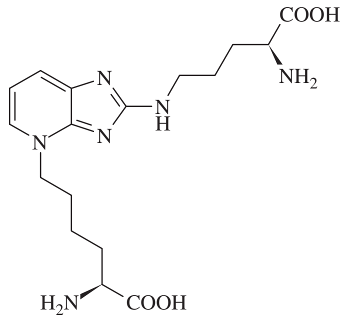


4-239, 3DG-imidazolone derived from arginine

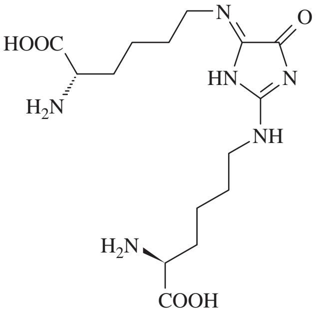


4-240, PIO

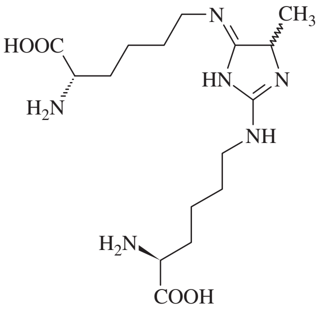
The result of cross-linking between the side chains of lysine and arginine are usually derivatives of imidazole. Pentosidine (**4-241**), 2-ammonio-6-{2-(4-ammonio-5-oxido-5-oxopentyl)-amino-3*H*-imidazo[4,5-b]pyridin-4-ium-4-yl}hexanoate, is a fluorescent compound that is formed on reaction of pentoses (and possibly of ascorbic acid) with lysyl and arginyl residues in proteins. It was detected in plasma β_2 microglobulin from patients with haemodialysis-related amyloidosis (a disease that results from the abnormal deposition of the protein amyloid). Pentosidine is normally present in acid hydrolysates of tissue rich in collagen. Other cross-linkages, which include side chains of lysine and arginine, are structures that are formed by reactions with glyoxal. An example of these compounds is 2-amonio-6-({2-[(4-amonio-5-oxido-5-oxopentyl)amino]-4,5-dihydro-1*H*-imidazol-5-ylidene}amino)hexanoate, or for short GODIC (**4-242**). By analogy, the reaction of methylglyoxal yields 2-amonio-6-({2-[(4-amonio-5-oxido-5-oxopentyl)amino]-4-methyl-4,5-dihydro-1*H*-imidazol-5-ylidene}amino)hexanoate, known as MODIC (**4-243**), 3-deoxyglycosuloses give rise to 2-amonio-6-({2-[(4-amonio-5-oxido-5-oxopentyl)amino]-4-(2,3,4-trihydroxybutyl)-4,5-dihydro-1*H*-imidazol-5-ylidene}amino)hexanoate, or DODIC (**4-244**), and the product of hexoses is 2-amonio-6-({2-[(4-amonio-5-oxido-5-oxopentyl)amino]-6,7-dihydroxy-4,5,6,7,8,8a-hexahydroimidazo[4,5-b]azepin-4-yl}hexanoate, known as glucosepane (**4-245**), the major protein cross-link of the senescent human serum albumin and lens protein. The levels of these adducts in hydrolysed foods (biscuits, cereal bars, boiled egg white) are up to tens of milligrams per kilogram.



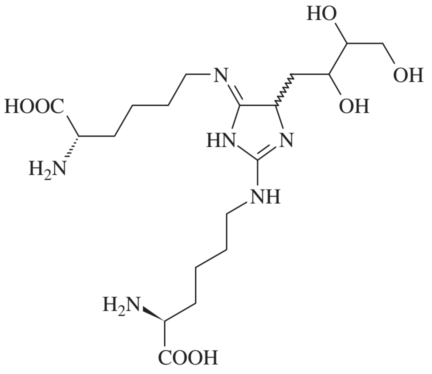
4-241, pentosidine



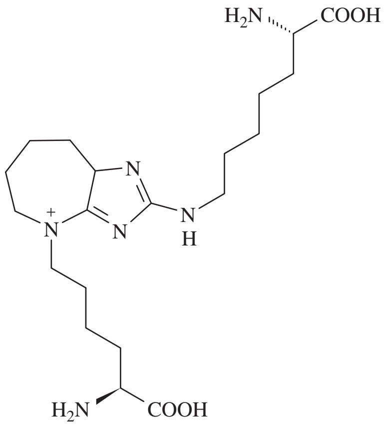
4-242, GODIC



4-243, MODIC



4-244, DODIC



4-245, glucosepane

An organism usually has a number of protective mechanisms that may limit and minimise the adverse consequences associated with AGEs formation. There are both chemical and biochemical processes, including enzymatic and immune responses. Suppression of glycation reactions and repair of glycated proteins in physiological systems are facilitated by a group of enzymes including glyoxalases, aldehyde reductases, aldehyde dehydrogenases, amadoriases and fructosamine 3-phosphokinases. The enzyme system of glyoxalase I and glyoxalase II, which plays a significant role in delaying the aging of tissues, metabolises the major AGEs precursors, such as glyoxal and methylglyoxal, and thus prevents glycation of cellular and extracellular proteins.

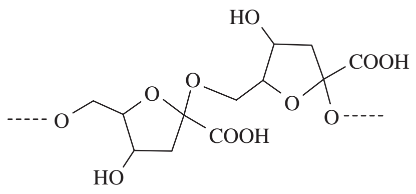
The risk of physiological AGEs is also reduced by a number of substances occurring in foods. Antioxidants (flavanones and other phenolic compounds, vitamin E, ascorbic acid and carotenoids) may inhibit oxidative stress in tissues and thus interfere with the formation of AGEs. Similar protective antiglycation effects are found with the elements Se, Zn, Cu and Mn, which are part of the redox enzyme systems. In addition to agents such as aminoguanidine, the α-reactive dicarbonyl precursors may also decompose thiamine and its derivatives. Foods high in fat (roasted almonds and walnuts, butter and mayonnaise) and protein (heat processed meats and cheeses) have 30 times and 12 times higher levels, respectively, the AGEs (expressed in CML content) of foods that are rich in carbohydrates (bread and other cereal products, fruits and vegetables). The important factor influencing the formation of AGEs is high temperature in processes such as grilling, frying or roasting. The dietary intake of AGEs substantially exceeds their amount generated physiologically. Usually less than 10% AGEs is absorbed from the dietary intake. Modified proteins are more resistant to proteolysis, and some AGEs even inhibit intestinal proteases. AGEs that are absorbed, are mainly glycated amino acids (adducts released by protein hydrolysis), which are rapidly excreted in urine in healthy people. Peptides that contain AGEs are eliminated from the body in the same way as xenobiotics. The relationships with the increase in the physiological levels and other negative influences have not yet been demonstrated for dietary AGEs and their interactions and reactions in the colon have not yet been adequately documented.

A considerable, but so far little explored technological potential of glycation reactions involves changes in the functional properties of proteins (formation of emulsions, foams, gels, changes in solubility, stability or improvement of taste). The disadvantage of these reactions is the certain, but usually negligible reduction of the nutritive value of proteins. Unlike acetylation, succinylation or other chemical methods used to improve the functional properties of proteins, the Maillard reaction occurs spontaneously during thermal processes without the addition of foreign substances. For example, increased solubility and emulsifying properties of proteins depend on the introduction of a limited number of hydrophilic sugar structures that are in contact with the aqueous phase, while the hydrophobic protein areas are oriented to the lipid phase. Increased stability of proteins may be partly related to the blocking of lysine and arginine residues that are attacked by proteolytic enzymes.

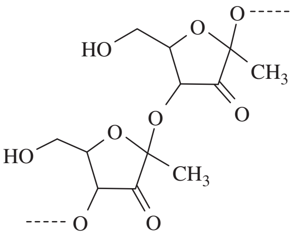
4.7.5.7 Melanoidins

The compounds produced in the first two phases of the Maillard reaction are usually colourless compounds, which are often collectively called **premelanoidins**. Different coloured compounds are formed mainly in the final stage of this reaction. Some of these are low molecular weight substances (usually with a relative molecular weight below 1000 Da), and other coloured substances known as **melanoidins** have relative molecular weights higher than 1000 Da. They have characteristic physico-chemical properties, such as solubility and antioxidant activity.

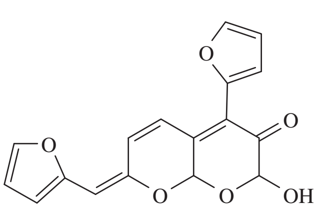
Coloured pigments that do not contain nitrogen are formed from various decomposition products of sugars. They can be formed, for example, by dehydration of the individual oligomers produced from 3-deoxy-D-*erythro*-hexos-2-uloses (the corresponding acids formed by their oxidation; **4-246**) or oligomers of 1-deoxy-D-*erythro*-hexo-2,3-diulose (**4-247**). In mixtures of pentoses and hexoses with amino acids, low molecular weight chromophores have been characterised, formed from the transformation products of 3-deoxyglycos-2-uloses (e.g. furan-2-carbaldehyde) and from reactive sugars fragments (**4-248** and **4-249**). The significant degradation product of 1-deoxyhexo-2,3-diuloses diacetylformoin (Figure 4.39) is another precursor of nitrogen free chromophores, but only if primary amino acids are not present. Otherwise its reaction with amino acids gives rise to pyrrolinone reductones that react via a methyl group with carbonyl compounds (such as furan-2-carbaldehyde) to form coloured compounds (Figure 4.99). Some other coloured reaction products are described in Section 4.7.1.1.4.



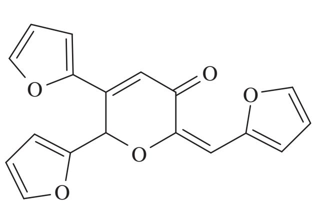
4-246, oligomer of 3-deoxy-D-*erythro*-hexos-2-ulose



4-247, oligomer of 1-deoxy-D-*erythro*-hexo-2,3-diulose



4-248, coloured pyrano[2,3-*b*]pyran-3-one derivative



4-249, coloured 6*H*-pyran-3-one derivative

While low molecular weight chromophores arise mainly in systems with free amino acids, the coloured Maillard reaction products with proteins are almost exclusively macromolecular substances,

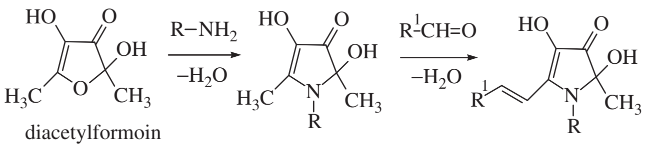
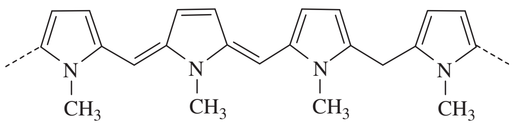
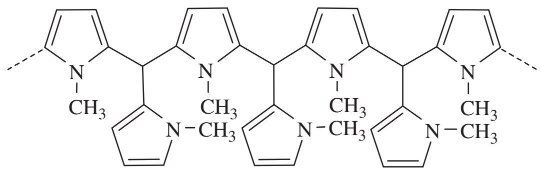


Figure 4.99 Formation of chromophores from diacetylformoin, aldehydes and amino acids.

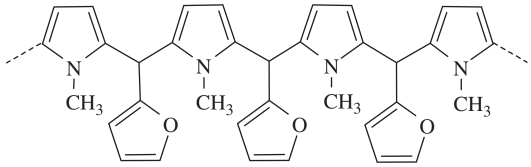
unless there is subsequent proteolysis. Current hypotheses include two main types of high molecular weight pigments. The first type of formation assumes the condensation of low molecular weight Maillard reaction intermediates to form the coloured substances. The structure of melanoidins resulting from the condensation of monomeric units (especially heterocyclic Maillard reaction intermediates) has not been described satisfactorily. For example, melanoidins may also arise in reactions of hydroxylated 1,4-dihydropyrazines formed by oxidation and hydration of pyrazinium radicals (Figure 4.84) or by their condensation with aldehydes (especially with furan-2-carbaldehyde). Other degradation products of pentoses and hexoses, such as *N*-substituted pyrroles or *N*-substituted pyrrole-2-carbaldehydes, similarly form easily coloured products. Coloured oligomers formed from 2-hydroxymethyl-1-methylpyrrole (**4-250**) by condensation of 1-methylpyrrole with 1-methylpyrrole-2-carbaldehyde (**4-251**) or furan-2-carbaldehyde (**4-252**) have been identified. The other two types of polymers are formed by nucleophilic addition of amino groups of Amadori compounds to carbonyl compounds and by subsequent dehydration. The resulting polymer is oxidised with the formation of a system of conjugated double bonds. Decarboxylation and elimination reactions transform these polymers into other conjugated systems (Figure 4.100).



4-250, 2-hydroxymethyl-1-methylpyrrole polymer



4-251, 1-methylpyrrole and pyrrole-2-carbaldehyde polymer



4-252, 1-methylpyrrole and furan-2-carbaldehyde polymer

The second method of melanoidin formation, which also leads to protein oligomers, is more typical for foods. This reaction assumes covalent bond formation of transformation products of sugars on the side chains of proteins, especially of bound lysine, arginine or cysteine, to form chromophore structures. Some of the previously mentioned AGE structures, such as pentosidine (**4-241**) are coloured. Other chromophores include substances that are formed in systems containing furan-2-carbaldehyde. The lysyl residue in a peptide chain reacts in a neutral solution with furan-2-carbaldehyde, forming chromophore (**4-253**). Also described are

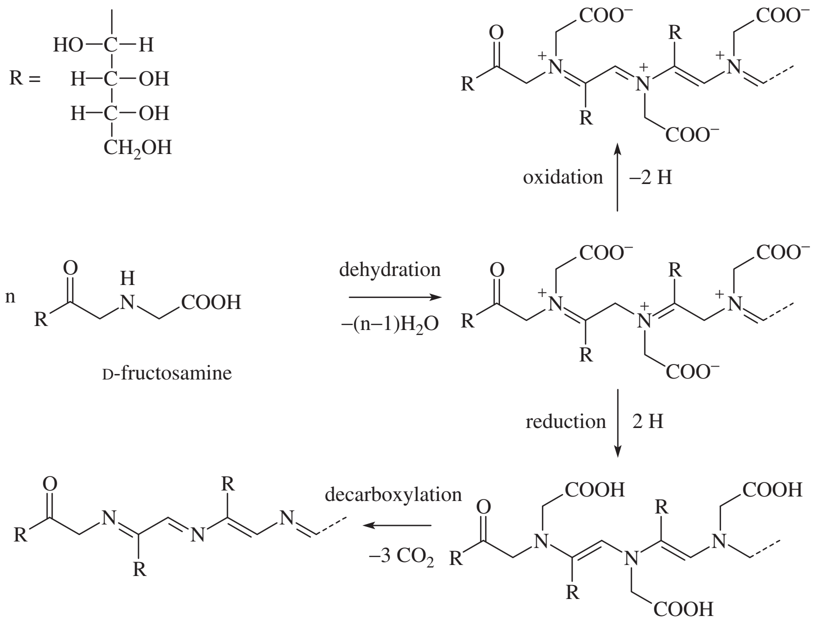
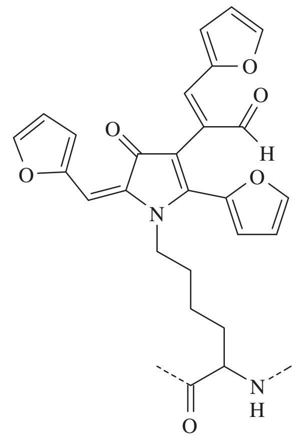
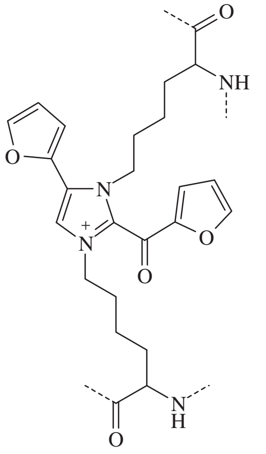


Figure 4.100 Expected structure of glucose–glycine pigments.

chromophores formed between two lysyl residues (**4-254**). Reaction of the guanidyl group of an arginine residue with glyoxal and furan-2-carbaldehyde yields the chromophore (**4-255**). Among the structures that cause protein cross-linking is the radical 1,4-bis(5-amino-5-carboxy-1-pentyl)pyrazinum cation abbreviated crosspy (**4-256**) bound to two lysyl residues of the peptide chains. Its formation is given in Figure 4.85. This structure is also considered one of the key precursors of melanoidins in bread crust and in roasted coffee. It is also possible that the chromophores of low molecular weights are only captured by the polymer via physical bonds instead of covalent bonds. The colour is sometimes attributed to the presence of free radicals stabilised in the structure of Maillard polymers. Other coloured structures may represent complexes with metal ions that create chromophores via chelation from colourless organic compounds, such as reductones.



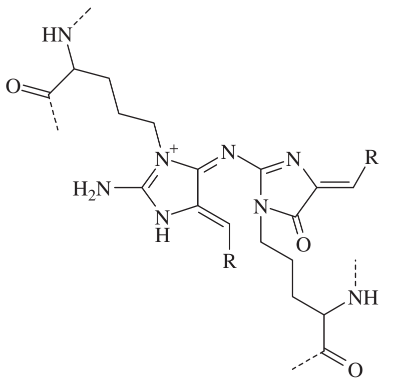
4-253, chromophore bound to protein through a lysine residue



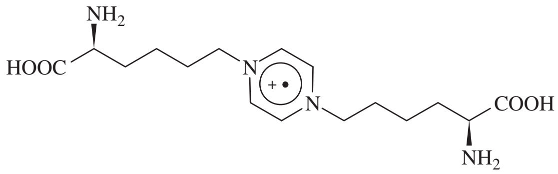
4-254, chromophore bound to a protein through two lysine residues

4.7.5.8 Antioxidant properties of reaction products

The important property of some melanoidins is their antioxidant activity. Antioxidant activity is highly dependent on the



4-255, coloured products of glyoxal and furan-2-carbaldehyde (R = furyl) with two arginine residues



4-256, 1,4-bis(5-amino-5-carboxy-1-pent-1-yl)pyrazinium radical cation

nature of the reactants from which melanoidins are formed. Some melanoidins also exhibit prooxidant properties.

The antioxidant properties of some reaction products of reducing sugars with amino acids and with other amino compounds (ammonia, amines) have been known for several decades, and have been used in industrial processes. Amino acids are less active antioxidants, but sugars and their transformation products do not show these properties. For example, the addition of glucose and certain amino acids (particularly glycine, valine and lysine) to the dough in the production of biscuits increased the stability of the fat towards autoxidation. The same effect is seen for the addition of reaction products of glucose with histidine in sausages stored at refrigerated temperatures. Heating milk with a mixture of glucose and histidine increases the oxidative stability of the milk after drying, but reduces the available lysine content and can bring about discolouration during storage. Products of the Maillard reaction exhibit synergistic effects in the presence of some antioxidants. For example, products arising from D-xylose and ammonia can increase the antioxidant effects of tocopherols.

The chemical principle behind the antioxidant properties of Maillard reaction products is currently not well understood. It is assumed that these properties show both low molecular weight products and high molecular weight melanoidins. As the structure of melanoidins has not been clarified satisfactorily, it is difficult to explain the chemical nature of their antioxidant activity. The active structures are probably reductones and aminoreductones, bound in melanoidin molecules that reduce the products of autoxidation. One of a few identified reductone structures in real food melanoidins is 2,4-dihydroxy-2,5-dimethyl-1-(5-acetamino-5-methoxycarbonylpentyl)-3-oxo-2*H*-pyrrole bound by a peptide bond. This so-called pronyl-L-lysine (pyrrolinone

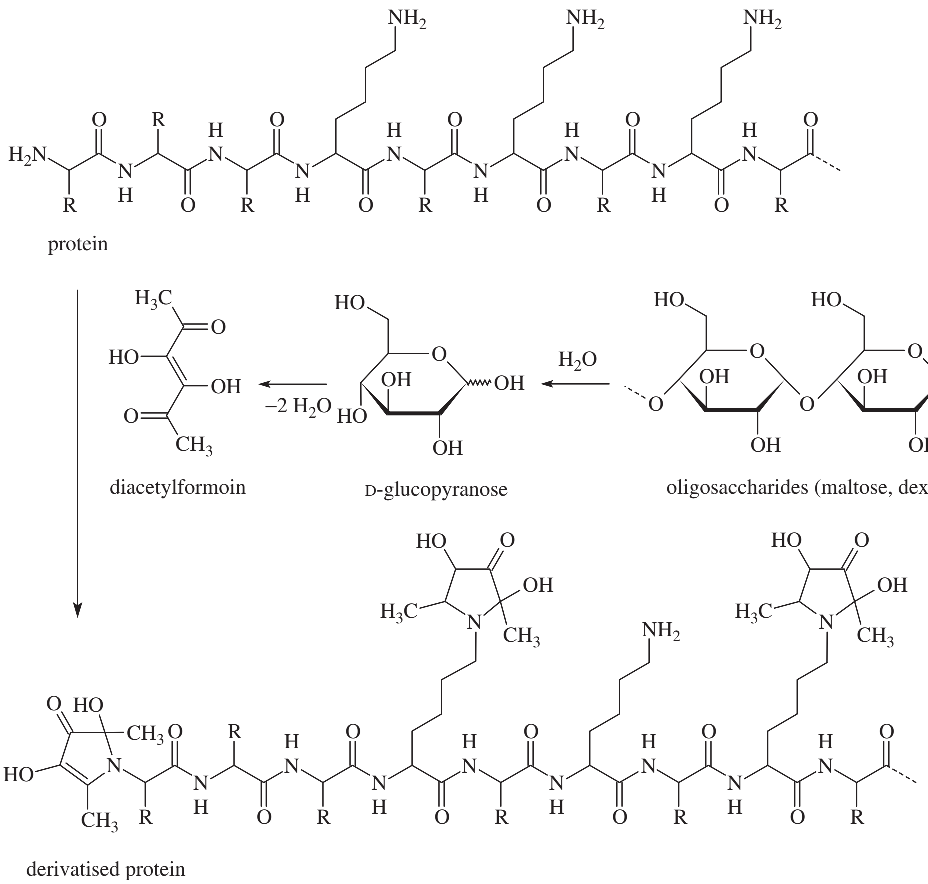
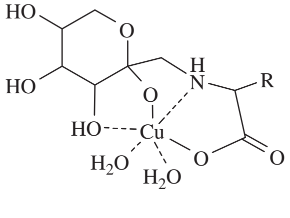


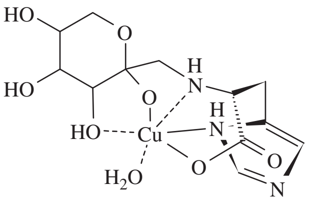
Figure 4.101 Formation of bound pronyl-L-lysine in bread crust.

reductonyl lysine) contributes significantly to the antioxidant properties of bread crust, where it is found in concentrations of about 60 mg/kg. Its amount depends on the type of bread dough, the pH during dough fermentation and conditions during baking. Pronyl-lysine forms in the reaction of diacetylformoin (dehydration product of D-glucose via 1-deoxy-D-*erythro*-hexo-2,4-diulose) with ε-amino groups of lysyl residues and also, by analogy, with the *N*-terminal lysyl residues of the peptide chain (Figure 4.101).

In addition to reductones, structures capable of binding metal ions that act as oxidation catalysts also have significant antioxidant activities. Although amino acids can bind metals to coordination compounds (see Section 6.2.2.1), metal chelates (M^{2+}) with Amadori compounds (**4-257**) have considerably higher stability and therefore higher antioxidant activity. The increased stability is due to additional metal ion binding by C-2 and C-3 hydroxyl groups. Significant amounts of N^{α}-(1-deoxy-D-fructos-1-yl)-L-histidine were found in dried tomatoes and some other dried products (**4-258**).



4-257, general structure of Amadori compound/metal complex



4-258, N^{α}-(1-deoxy-D-fructos-1-yl)-L-histidine/Cu^{2+} complex

The highest antioxidant activity is seen in reaction products formed from sugar degradation products (such as methylglyoxal and 1,3-dihydroxyacetone) and from dehydroascorbic acid. Less active as antioxidants are the reaction products of pentoses, and even less active reaction products are formed from hexoses. The products of sugars with a basic amino acid (arginine, lysine and histidine) show higher antioxidant activity than products of other amino acids. Products of arginine and histidine with xylose are effective as antioxidants. Glutamic acid reaction products with glucose or fructose do not exhibit antioxidant properties. Reaction conditions play an important part. Antioxidant activity of the

reaction products generally increases with increasing pH, and with increasing weight ratio of the amino compound to sugar.

4.7.5.9 Nutritional and toxicological aspects

The consequences of the Maillard reaction are desirable changes to the organoleptic properties of foods, such as the formation of the typical aroma, taste and colour. However, some adverse changes can also appear, the most serious of which is the emergence of unusual taste and odour, undesirable colour (e.g. in dried foods) and reduced nutritional value. The decrease in nutritional value is caused by the actual loss of essential amino acids by irreversible reactions (e.g. in Strecker degradation), by binding of amino acids to unusable complexes and covalent compounds and by decreased protein digestibility due to resistant cross-links. The non-enzymatic browning reactions mainly affect the ε-amino group of lysine and sulfur containing amino acids.

The initial phase of the Maillard reaction, in which Schiff base stabilising by cyclisation is effective, does not negatively affect the nutritional value of foods, because both of these reaction intermediates are easily hydrolysed to the original components. The next stage of the Maillard reaction, which is the rearrangement of *N*-glycosylamine to aminodeoxysugar, results in a reduction of the food's nutritional value. For example, aldoses yield the so-called Amadori compounds (1-amino-1-deoxyketoses), which are the main bound and unusable forms of lysine in foods. The reduction of the nutritional value during the Maillard reaction occurs primarily in heat-stressed foodstuffs with low water content (drying, baking, frying and roasting). Bread baking, for example, results in a 10–15% loss of total lysine, and of up to 70% of lysine from the bread crust. Higher losses of lysine also occur during the drying of milk, where the loss can reach up to 30%, depending on the drying technology.

Melanoidins in foods high in saccharides, such as bread crust or coffee, can have particular biological functions of fibre, including the growth of desirable intestinal microflora (prebiotic effect). Melanoidins derived mainly from proteins do not show these properties.

As products of the Maillard reaction, numerous toxic substances can also be formed that exhibit clastogenic, mutagenic and carcinogenic effects. In this respect, various pyridoimidazoles, pyridoindoles and tetraazafluoranthenes occupy significant positions (see Section 12.2.1). Some products of the Maillard reaction are secondary amines. Reaction with nitrous acid or with nitrogen oxides may transform these compounds into mutagenic and carcinogenic *N*-nitroso compounds. In addition to these compounds, some Maillard reaction products show mutagenic activity, such as glyoxal, methylglyoxal and 5-hydroxymethylfuran-2-carbaldehyde.

4.7.5.10 Factors influencing reactions

The practical application of the Maillard reaction during food processing requires it to be under control in order to suppress side reactions and highlight the desirable or beneficial reactions. The main factors influencing the course of the Maillard reaction, which can be used for its control during food processing, are:

- temperature
- reaction time
- pH
- water activity
- type of reactants
- availability of reactants.

Owing to the complexity of the Maillard reaction, its optimisation is not an easy task. Optimisation is also impeded by the fact that the individual factors, such as temperature, water activity and pH, do not act in isolation, but can influence each other. The study of the influence of individual factors on the reaction is considerably more difficult, and the interdependence of the various factors is often the cause of conflicting and contradictory results obtained in different situations.

The effect of temperature on chemical reactions is expressed as the activation energy of reaction (higher activation energy means that the reaction rate depends more on temperature). The values of activation energy of the Maillard reactions vary over a wide range from 10 to 160 kJ/mol. The activation energy is also highly dependent on water activity. For example, at low water activities the activation energy of Amadori compound formation increases rapidly, while at medium and higher values of water activity, the activation energy is virtually independent of water activity. The activation energy (especially the activation energy of brown pigment formation) is also dependent on pH. For example, the activation energy of the formation of pigments in a lysine–glucose system increases with decreasing pH. Temperature affects not only the Maillard reaction rate, but also often the reaction mechanisms leading to the final products. A typical example is the formation of aromatic compounds, as the same reaction mixtures heated to different temperatures can produce very different flavour-active compounds. Higher temperatures generally lead to extensive fragmentation of reactants and a more diverse mixture of reaction products.

Water activity also significantly influences the formation of the brown colour. At high values of water activity, due to dilution of reactants, the reaction rate is very low. With decreasing water activity, the reaction rate increases, especially at medium water activity values (0.3–0.7), and reaches a maximum. Further reduction in water activity leads to a further decrease of reaction rates (see Section 7.9.2). Very low values of water activity can lead to the reaction stopping due to lack of mobility of the reactants. The value of water activity at which the Maillard reaction rate reaches a maximum can be influenced by the addition of glycerol or ethylene glycol. While the addition of these agents in systems with higher water activity reduces the reaction rate (dilution of reactants), their addition to systems with low water activity increases the reaction rate (higher mobility of reactants).

The Maillard reaction is highly dependent on pH (the reaction is usually accompanied by a decrease in pH as a result of formation of various carboxylic acids). The Maillard reaction rate generally increases with an increase in pH value. Brown colour formation in reactions of sugars with various amino acids increases with pH and reaches a maximum in the pH range 9–10 (the system glucose–lysine produces about 500 times higher amounts of pyrazines in pH 9 in comparison with pH 5). As with temperature, pH affects not only the Maillard reaction rate (and, therefore, the quantitative composition of the reaction mixture), but also its qualitative composition. A typical example is the decomposition of ketosamines, where pH is a very important factor in determining whether the decomposition takes place by 1,2- or 2,3-enolisation. While the 1,2-enolisation is favoured in sufficiently acidic media that allow protonisation of the Amadori compound nitrogen, during 2,3-enolisation the Amadori compound must be partially deprotonised. Therefore, 2,3-enolisation dominates in alkaline solutions and under non-aqueous conditions.

The effect of reaction partners on the rate of the Maillard reaction in the early stages has been already discussed (see Section 4.7.5.1). The rate of addition is mainly related to the pK_a value of amino compound, which determines the concentration of reactive species at a certain pH of the reaction mixture. The fastest reacting free amino acid in a wide range of pH values is lysine; the least reactive are aliphatic hydrophobic amino acids valine, leucine and isoleucine. Under normal conditions (pH 3–8) oligopeptides (mainly dipeptides) are more reactive than the corresponding amino acids, because their pK_a values are substantially lower. Reactivity of peptides is highly dependent on their primary structure. For example, dipeptides with *C*-terminal glutamic acid are more reactive. Another parameter of comparative reactivity of compounds is their disposition to the formation of aromatic or coloured products. For example, lysine, glycine or tryptophan produce a very intense colour with sugars, but the acidic, sulfur and hydroxyamino acids contribute little to the browning reaction. Furan-2-carbaldehyde and glycolaldehyde belong to the most effective colour precursors in the presence of amino acids and common carbonyl intermediates such as pyrrole-2-carbaldehyde, methylglyoxal or glyoxal similarly have considerable potential. Amadori products provide fewer colours than the starting hexoses and pentoses. 5-Hydroxymethylfuran-2-carbaldehyde or acetoin are insignificant reaction products as pigment precursors.

Some importance is attributed to the Maillard reaction during the germination of plant seeds due to the increased availability of free amino acids and sugars. Several intermediates of the Maillard reaction are apparently applied as stimulants of germination.

4.7.5.11 Reaction inhibition

Taking into account that the Maillard reaction is not always desirable during the processing and storage of foods, a range of possibilities of its inhibition have been studied. Inhibition of the Maillard reaction consists mainly of creating conditions unfavourable to its progress. Knowing the factors that influence the course of the Maillard reaction, it is clear that a reduction of its rate or inhibition of this reaction can be achieved in many ways, but not all of them are applicable in practice. The choice of inhibition method therefore greatly depends on the specific food and its processing technology. Most often the following inhibition methods are employed:

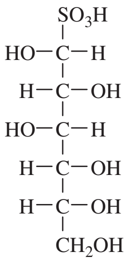
- elimination of one of the reaction partners
- water content adjustment
- decreased temperature
- shorter heating period
- pH adjustment
- addition of substances inhibiting or slowing down the reaction.

Removal of glucose has been used successfully in the production of powdered (dried) whole eggs and egg whites. Glucose is oxidised to inactive gluconic acid by the addition of a yeast preparation with glucose oxidase activity, or by the addition of glucose oxidase enzyme. Simultaneous removal of oxygen slows down the autoxidation reaction.

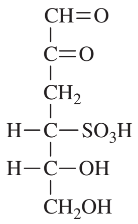
Limiting the extent of the Maillard reaction in the production of dried foods is often achieved by reducing the temperature currently used and drying time to a minimum. This can be done by frequently turning the food, or drying it in the thinnest layer possible. It is important to reduce the temperature at which the Maillard reaction takes place quickly, especially when the food has a critical water content (water activity). It is also necessary to ensure that the finished dried product is not heated unnecessarily. For example, during spray drying of milk it is necessary to avoid heating the dried powder above 60 °C. Reducing the heating time is employed, for example, in the production of jams. The use of small production batches can shorten the time of heating by up to one third, which has a significant positive effect on the sensory quality of the product. While products produced in large batches tend to have a reddish brown colour and a caramel-like flavour, the product prepared by the same formula, but in smaller batches, has a deep red colour and typically a strawberry flavour. Reducing the heating time also indirectly influences the course of the Maillard reaction by lowering the amount of glucose produced by saccharose inversion.

Inhibition of the Maillard reaction in certain foods can also be achieved by the addition of sulfur dioxide or hydrogen sulfites (bisulfites). It is assumed that the inhibition occurs due to hydrogen sulfite addition to the carbonyl group of sugar, which is then blocked and cannot react with amino compounds. In a weakly acidic medium and at normal temperatures, glucose yields the corresponding 2-hydroxysulfonic acid, the systematic name of which is D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexanesulfonic acid (**4-259**). In a neutral solution 4-sulfohexos-2-ulose (3,4-dideoxy-4-sulfo-D-*glycero*-hexos-2-ulose) is produced, shown in **4-260**. 4-Sulfohexos-2-ulose can be further transformed into isomeric acids by oxidation or benzilic acid rearrangement. Inhibition of reactions with sulfur dioxide or hydrogen sulfides in the presence

of ascorbic acid or pentose yields, analogously, 3,4-dideoxy-4-sulfopentos-2-uloses.



4-259, D-*glycero*-D-*ido*-1,2,3,4,5,6-hexahydroxyhexane sulfonic acid



4-260, 3,4-dideoxy-4-sulfo-D-*glycero*-hexos-2-ulose

The effectiveness of inhibition by hydrogen sulfites is undoubtedly related to their ability to react with a wide range of products produced at all stages of the Maillard reaction. Enzymatic browning reactions are also inhibited. Carbon dioxide also has a preservative function, or may act as an antioxidant. Inhibition of the Maillard reaction can also be achieved by adding sulfur-containing amino acids such as cysteine; however, due to the possibility of degradation of sulfur amino acids and formation of unpleasant sulfur-containing flavour-active degradation products, the practical application of this inhibition is only possible in exceptional cases.

4.7.5.12 Significance for food technologies

Most food technologies have a long tradition, and control of the Maillard reaction is based on extensive empirical experience rather than on scientific knowledge gained by systematic research. When new technologies have to be introduced, food processing technologists are faced with the task of optimising these technologies in order to achieve the required product quality.

When the pre-existing technology stands to benefit from improvements that enhance and complement its performance-in-use, the required product quality has to be at least comparable to the product quality obtained by the traditional technology (such as microwave cooking versus traditional cooking). Optimisation of the Maillard reaction in new technological processes is made more difficult by the fact that besides the lack of empirical experience, technologists must often establish new parameters, which previously influenced the course of the Maillard reaction only marginally. For this reason, they are forced to use their scientific knowledge for control of the Maillard reaction to a greater extent than in the conventional technology. The relatively new technologies, where the influence of the Maillard reaction is not fully optimised, are:

- extrusion
- microwave cooking
- infrared heating.

4.7.5.12.1 Roasting

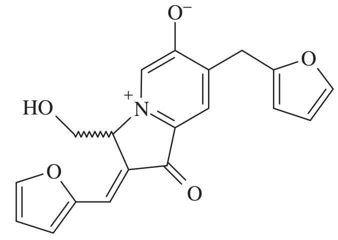
One of the traditional processes, in which the Maillard reaction is very intense, is roasting (manufacture of cocoa and coffee and nut roasting). Roasting affects not only colour, but also mainly the odour and taste of the processed raw materials.

During the roasting of cocoa beans, hundreds of volatile compounds are created, of which more than 400 have already been identified. The mechanisms of formation of many of these substances have been satisfactorily explained. It was also found that the formation of volatiles is not only affected by the conditions of roasting, but to a large extent also by fermentation prior to roasting, which leads to the release of amino acids and reducing monosaccharides. Not all volatile products generated during roasting of cocoa beans, however, are important components of cocoa aroma.

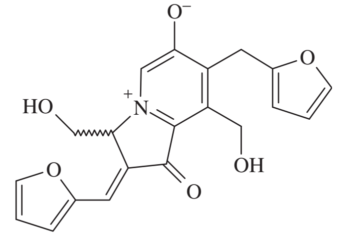
Roasting of green coffee beans also creates hundreds of volatile substances. Even more compounds have been identified in roasted coffee than in roasted cocoa. Depending on the roasting conditions (particularly temperature and roasting time), roasted coffee with different sensory properties can be obtained. During the roasting process, reducing monosaccharides are decomposed in the first instance. In later stages of roasting, non-reducing oligosaccharides and polysaccharides, after cleavage to reducing sugars, are also involved in the Maillard reaction. At lower roasting temperatures, the rate of release of reducing sugars from polysaccharides and oligosaccharides is higher than their decomposition. Coffee roasted in this way has a lighter colour and contains up to 1% of glucose and fructose. At high temperatures, rapid decomposition of reducing sugars occurs and higher amounts of polymeric brown pigments are formed. Reducing sugars are also partially decomposed to acids under these conditions and the acids contents grow. Thus, roasted coffee normally contains only traces of reducing sugars, has a dark colour and a slightly sour taste. In recent years the so-called fast roasting process has also been introduced (at temperatures higher than 230 °C), in which the saccharose content in coffee beans sharply decreases, but the loss of polysaccharides is substantially lower than during the conventional roasting, which is reflected in reduced acidity.

The most famous coffee substitute (surrogate) is undoubtedly chicory root, which contains a considerable amount of inulin. Inulin is accompanied by free reducing sugars (glucose and fructose). Roasting chicory root gradually reduces the amount of inulin, and reducing sugars and fructose produced from inulin are subsequently degraded to dark caramelisation products (see Table 4.10).

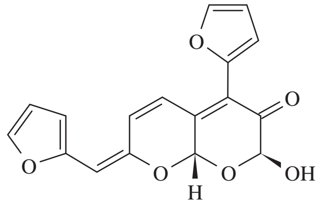
The roasting processes produce a number of taste-active substances, bitter compounds in particular. Unlike the odour-active substances created by the Maillard reaction, knowledge of the bitter compounds is rather limited. Most of the identified substances were found in model reaction mixtures. It is known that pentoses and α-amino acids (such as xylose and alanine) produce intensely bitter substances identified as (*E*)-7-(2-furylmethyl)-2-(2-furylmethylidene)-3-(hydroxymethyl)-1-oxo-2,3-dihydro-1*H*-indolizinium-6-olate (quinizolate, **4-261**) and (*E*)-7-(2-furylmethyl)-2-(2-furylmethylidene)-3,8-bis(hydroxymethyl)-1-oxo-2,3-dihydro-1*H*-indolizinium-6-olate (also known as homoquinizolate, **4-262**). Diastereoisomers of 2*H*,7*H*,8a*H*-pyrano[2,3-*b*]pyran-3-one (**4-263**) have a taste reminiscent of capsaicin. Bitter tasting compounds in reaction mixtures of hexoses with proline are a spirodiolone derivative **4-264**, bispyrrolidinohexose reductones (**4-225**) and also cyclopenta[*b*]azepin-8(1*H*)-ones (**4-226**). Other products, such as 2,5-dimethyl-4-(1-pyrrolidinyl)-2*H*-furan-3-one (**4-266**) or 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-2-one (**4-265**), cause afterimage cooling and a refreshing taste in the mouth. These substances have similarly been found in roasted malts. Mixtures of hexoses with alanine yield an inner salt of 1-(1-carboxyethyl)-5-hydroxy-2-(hydroxymethyl)pyridinium (**4-267**), which increases the intensity of the sweet taste perception. For example, similar pyridinium betaines were found in beef broth.



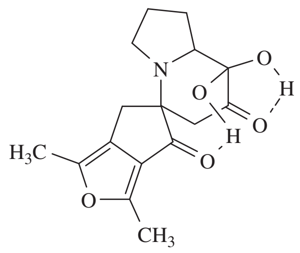
4-261, quinizolate



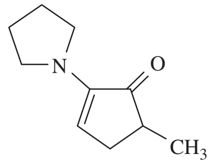
4-262, homoquinizolate



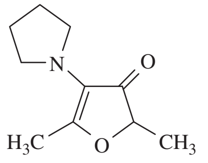
4-263, 2*H*,7*H*,8a*H*-pyrano[2,3-*b*]pyran-3-one



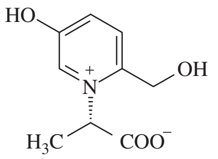
4-264, spirodiolone derivative



4-265, 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-2-one



4-266, 2,5-dimethyl-4-(1-pyrrolidinyl)-2*H*-furan-3-one



4-267, 1-(1-carboxyethyl)-5-hydroxy-2-(hydroxymethyl)pyridinium

4.7.5.12.2 Boiling, baking and frying

Besides roasting, many other processes exist where the Maillard reaction is desirable (e.g. cooking, baking and frying). The Maillard reaction can also have negative consequences, such as a certain reduction of the nutritional value of food, production or mutagens, but the positive aspects of the Maillard reaction (especially the typical colour, smell and taste) significantly predominate. Examples include the formation of bread crust flavour and the flavour of cooked and roasted meat. Formation of aromatic compounds in the traditional processes of food production have always received, and continue to receive, considerable attention. Research is currently focused mainly on identifying compounds responsible for the characteristic odour and taste.

4.7.5.12.3 Drying

A typical example of a traditional technology in which the Maillard reaction manifests itself in a profoundly negative manner is the drying of milk, fruits and vegetables.

In milk and dairy products the intensity of the Maillard reaction is very high under certain conditions, due to the relatively

high concentration of lactose and the presence of thermolabile proteins (especially whey proteins). During drying, heat treatment of milk, and also during improper storage of milk powder, the non-enzymatic browning reactions destroy lysine almost exclusively. This amino acid becomes a limiting amino acid, although it was present in the original raw material in sufficient amounts. Losses of lysine greatly depend on the technology of the milk processing, as well as on appropriate practice of these procedures. The total amount of lysine bound to lactose in raw milk is very small, but the bound lysine is non-utilisable. During the traditional drying process using steam-heated drying cylinders, the lysine losses reached 10–30%, while spray drying losses only reach up to 3%. In the freeze-dried milk, practically no loss of lysine occurs.

The Maillard reaction in dried milk affects the originally free ε-amino group of L-lysine bound in proteins that reacts with lactose yielding the corresponding glycosylamine, which is fully utilisable as a source of lysine. Its rearrangement produces the non-utilisable protein bound Amadori compound (ε-*N*-deoxylactulosyllysine also known as 1-lysino-1-deoxylactulose). Hydrolysis of these modified proteins initially gives the free Amadori compound, which is subsequently degraded to furosine, pyridosine and lysine via the intermediate 1-lysine-1,4-dideoxy-D-*glycero*-hexo-2,3-diulose (Figure 4.102). Determination of pyridosine and furosine is used in practice for the tentative determination of the degree of damage in dried milk. The degree of damage to the milk powder, that is the amount of non-utilisable lysine, is very important information, especially for manufacturers of infant formulae.

The Maillard reaction is responsible for only about 20% of the losses of lactose in milk as the main cause of the losses (about 80%) is lactose isomerisation. Lactose isomerises even in the neutral medium of milk to produce lactulose (aldose–ketose isomerisation), which is accompanied by a small amount of epilactose (aldose–aldose isomerisation, known as epimerisation; Table 4.15). Lactulose in milk is a mixture of three isomers differing in the structure of bound fructose (β-furanose, α-furanose, β-pyranose in the ratio of about 75 : 10 : 15). It is commonly present in pasteurised, sterilised or otherwise heat-treated milk, and in milk products. UHT milk contains 50–750 mg/l of lactulose, while sterilised milk has higher lactulose content. The lactulose content clearly correlates with the negative organoleptic properties (cooked flavour, darker colour); therefore it is used as an indicator, which can differentiate pasteurised milk, sterilised milk and UHT milk. The lactulose content in condensed milk is up to 1%, which corresponds to about 10% of isomerised lactose. Lactulose is partly hydrolysed to fructose and galactose, and these monosaccharides isomerise to produce small amounts of glucose and tagatose, respectively. Isomerisation of lactulose (2,3-enolisation) yields 4-deoxy-D-*glycero*-hexo-2,3-diulose as an intermediate product and 3-deoxy-D-*glycero*-pentos-2-ulose and formic acid as the main final products. Dehydration of 3-deoxy-D-*glycero*-pentos-2-ulose yields small amounts of furfuryl alcohol.

Amadori compounds have been identified in various processed fruit and vegetable products, for example, in commercial carrot products (juices, baby foods, tinned and dehydrated carrots). While these products showed fairly low rates of amino acid modification (up to 5%), dehydrated carrots contained Amadori products corresponding to a lysine derivatisation of up to 58% and nearly 100% derivatisation of γ-aminobutyric acid. Besides the Amadori

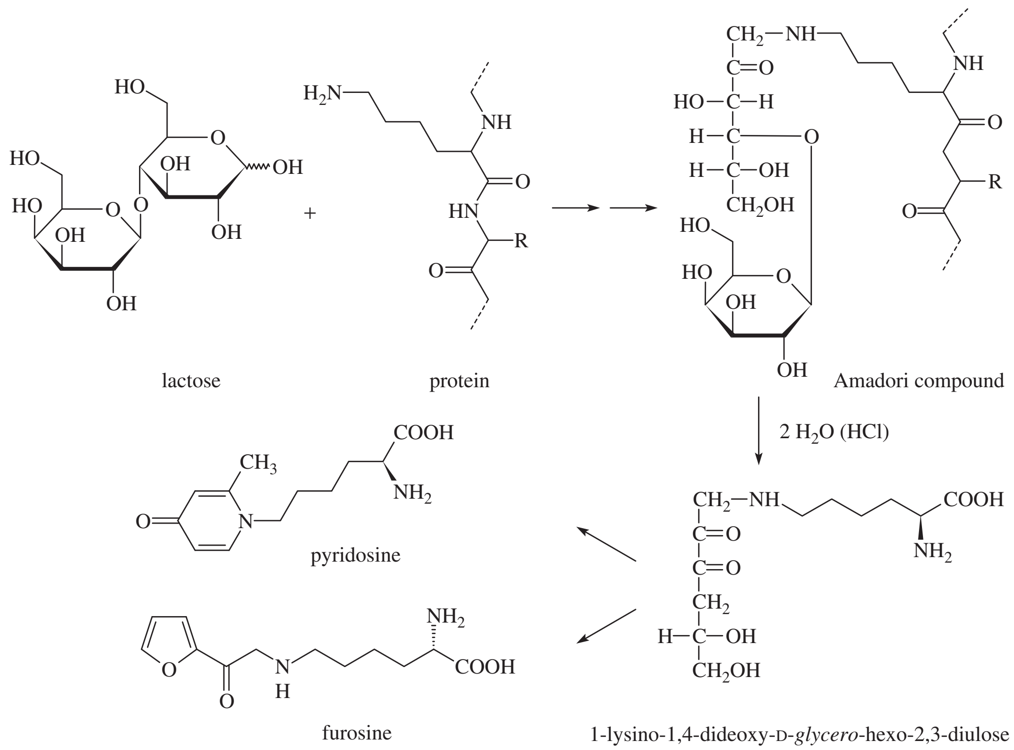


Figure 4.102 Formation of furosine and pyridosine from lactose and milk proteins.

products formed during the early stage of the Maillard reaction, the AGEs N^{ε}-(carboxymethyl)lysine (CML, **4-231**) and 6-(2-formyl-5-hydroxymethylpyrrol-1-yl)-L-norleucine (pyrraline, **4-232**) likewise occurred in all carrot products (except raw carrot).

4.7.5.12.4 Extrusion

Extrusion technology is now widely used with a range of food applications (such as production of biscuits and breakfast cereals). It is a relatively complex process in which food is exposed to comparatively high temperature, high pressure and shear forces for a short period of time. The quality of the final product depends on parameters such as the speed of performance of the extruder, screw speed and screw configuration (shape). High temperature and low water activity of the processed material produce favourable conditions for the Maillard reaction. The negative aspect is the loss of lysine, whereas a positive aspect is the formation of a desirable flavour of the product. Under unfavourable conditions, losses of lysine during extrusion can reach 40–50%. The losses can be minimised in various ways, such as by lowering the temperature, increasing the water content, using non-reducing sugars (saccharose) instead of reducing sugars and changing the geometry of the screw. For example, at a temperature of 210 °C, the increase of the moisture content from 13 to 18% results in roughly seven times lower loss of lysine during the production of biscuits. To maintain the lysine losses under 15% of the original lysine content, the recommended temperature is 180 °C and the water content in the material must be lower than 15%. The addition of reducing sugars is not recommended. Temperature and humidity are key parameters influencing the formation of aromatic compounds during extrusion. The most important fragrant substances in the extruded materials are pyrazines. Their content increases as the temperature increases to 160 °C, but at higher temperatures the levels of pyrazines decrease.

4.7.5.12.5 Microwave cooking

In recent decades, microwave cooking has undergone considerable growth. The highest temperature is achieved not only on the surface of the food (as in classical heating), but also inside the food. This can be a major disadvantage, because there is insufficient heating and drying of the surface, so the crust formation and colour and aroma development are insufficient. To reduce these shortcomings, several principles have been proposed that consist of:

- modifications of recipes
- changes of process parameters
- packaging technology.

An example of recipe modifications is the addition of artificial flavours or coating the surface of food by pre-mixes containing reducing sugars and amino acids. The actual process can be modified by the combination with another type of heating (such as grilling). Changes in packaging technology lie mainly in the use of packaging materials that are capable of absorbing the microwave radiation and can heat the surface of food with which they are in contact, such as a polyester film metallised with aluminium and glued onto paper. The temperature of the packaging material can be influenced by the thickness of the aluminium layer.

4.7.5.12.6 Infrared heating

Infrared heating is successfully used, for example, for cooking meat, and in the production of biscuits or bread. The main advantage in comparison with conventional methods is the shorter cooking time and energy savings. Sensory qualities of products obtained by conventional methods and by infrared heating are comparable; for example, bread forms a crust, but it is slightly thinner.

4.7.6 Caramelisation

Caramelisation of sugars is the process that produces brown to brown–black amorphous products of different composition that are referred to as **caramel**. A solution of caramel is called **couleur caramel** or **sugar couleur**. Caramel is produced by heating sugars or sugars with the addition of substances that accelerate caramelisation (catalysts) to temperatures higher than about 120 °C, typically 150–190 °C, but not exceeding 240 °C.

The raw materials for the production of caramel are mostly saccharose, glucose, fructose, glucose syrup or starch. During the discontinuous (batch) mode of caramel production, these materials are heated at 120–180 °C for 5–10 h in the presence of a catalyst. The choice of the catalyst depends on the intended use of the caramel. According to the technological process used (substances used in accelerating caramelisation), the following types of caramels can be distinguished:

- caramels with a positive electric charge (pH of isoelectric point is 4.0–7.0, in particular 6.0–7.0), produced by the addition of ammonia;
- caramels with a negative electric charge (pH of isoelectric point <3.0, usually around a value of 1.5), produced by the addition of ammonium sulfate or ammonium sulfite;
- caramels with no electric charge (produced in the presence of NaOH) known as spirit caramels.

Caramels are used to dye beer and other alcoholic beverages, soft drinks, vinegar, confectionery, bakery and meat products. Caramels with a positive electrical charge are particularly suitable for colouring beer, because in the presence of alcohol the positively charged colloidal particles of tannin are not precipitated and do not form hazes. Caramels with negative electric charges are used in the manufacture of soft drinks, which require caramel stability at low pH values. Spirit caramels are used for colouring alcoholic beverages such as rum, because they are soluble and stable in the presence of ethanol. Classification of sugar caramels and their uses are given in Table 4.37.

Table 4.37 Classification of sugar couleurs and their use.

Class		Couleur name	Additives	Use
I	CP	Caustic (plain, spirit)	Na_2CO_3, K_2CO_3, NaOH, KOH, H_2SO_4, acetic acid, citric acid	Spirits with high alcohol content
II	CCS	Caustic sulfite	SO_2, H_2SO_4, Na_2SO_3, K_2SO_3, NaOH, KOH	Malt bread, vinegar, bear, spirits, flavoured wines, mead
III	AC	Ammonium	NH_3, $(NH_4)_2SO_4$, Na_2CO_3, H_2SO_4, NaOH, KOH	Beer and other alcoholic beverages, acidic foods
IV	SAC	Sulfite ammonium	NH_3, SO_2, $(NH_4)_2SO_3$, Na_2SO_3, K_2SO_3, Na_2CO_3, K_2CO_3, NaOH, KOH, H_2SO_4	Acidic foods, non-alcoholic beverages

Caramel contains, in addition to high-molecular weight constituents, a variety of substances of low molecular weight, which are formed in the Maillard reaction. These substances largely include unreacted sugars, acids (mainly pyruvic acid), sugar anhydrides, furan and pyran derivatives and sugar fragments. Low molecular weight substances are the main precursors of reactions leading to the formation of brown polymeric melanoidins. Reactions taking place during the caramelisation of sugars are similar to those that occur during the Maillard reaction (such as dehydration, isomerisation and retroaldolisation). Unlike the Maillard reaction, caramelisation, with few exceptions, does not produce nitrogenous compounds. The only exception is caramelisation catalysed by ammonium salts or ammonia in which nitrogen compounds are formed, for example pyrazines, imidazoles and other heterocyclic compounds, but to a lesser extent than in the Maillard reaction.

By analogy with the Maillard reaction, some undesirable compounds can be similarly formed in caramelisation. Possible compounds are, for example, 4(5)-methylimidazole, 2-methylimidazole and 2-acetyl-4(5)-(*arabino*-1,2,3,4-tetrahydroxybutyl)imidazole (see Section 8.2.11.1.5) that may occur in caramels of the class III or IV (Table 4.37). Caramels with a low level of imidazoles can also be obtained by other technologies, for example by rapid continuous production processes or by extrusion of pregelatinised starch or dextrin at a temperature of 150–220 °C or they may be removed by ultrafiltration. 4(5)-Methylimidazole and 2-methylimidazole are classified by the International Agency for Research on Cancer (IARC) as 2B group agents that are possibly carcinogenic to humans. Internationally, JECFA (Joint FAO/WHO Expert Committee on Food Additives) has set the acceptable daily intake (ADI) of class II caramels to160 mg/kg body weight per day and those of class III and class IV to 200 mg/kg body weight per day and

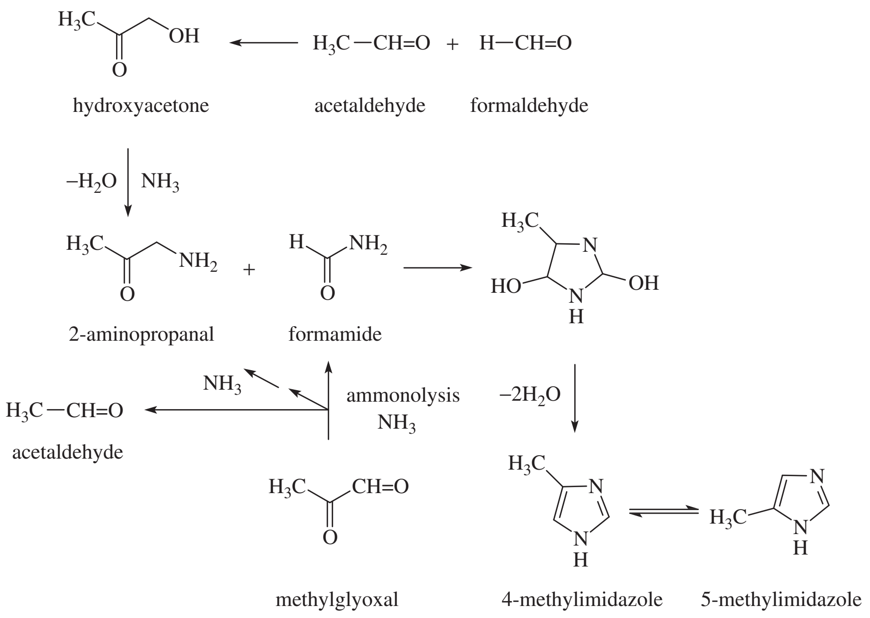


Figure 4.103 Formation of 4(5)-methylimidazole.

the European Commission has limited the 4(5)-methylimidazole content to 250 ppm (mg/kg). ADI of class I caramels has not been specified. For example, the levels of 4-methylimidazole found in commercial cola soft drinks ranged from 0.30 to 0.36 mg/l in 2011. The proposed formation mechanisms of 4(5)-methylimidazole from methylglyoxal and ammonia is given in Figure 4.103. Ammonolysis of methylglyoxal is proposed as the mechanism for forming acetaldehyde and formamide, which subsequently reacts with 2-aminopropanal arising as a product of hydroxyacetone and ammonia, to give 4(5)-methylimidazole. Hydroxyacetone also arises as a degradation product of sugars (and methylglyoxal).

5

Vitamins

5.1 Introduction

Vitamins are a group of low molecular weight organic substances present in minute amounts in natural foods that are essential to normal metabolism; insufficient amounts in the diet may cause symptoms of deficiency. Vitamins are synthesised almost exclusively by autotrophic organisms. Heterotrophic organisms synthesise vitamins only to a very limited extent (e.g. man can synthesise niacin from tryptophan) and or they can be obtained as exogenous substances mainly in food and in some cases through the enteric (intestinal) microflora. Vitamins are necessary for the regulation of human metabolism. They are not a source of energy or a building material, but mostly function as parts of enzymes catalysing biochemical reactions, although they have a number of other functions. Therefore, vitamins are often referred to as **exogenous essential biocatalysts**.[1]

Vitamins are substances with a variety of different chemical structures. In the past, before structures of all the vitamins were known and vitamin preparations were mixtures of various substances, so-called biological units (such as mice or chicken units) were introduced for quantitative purposes. The biological unit of a vitamin was related to the amount of that vitamin that produced a physiological effect to the respective animal within a given time. Later, so-called international units (IU) were derived, related to a particular vitamin weight. International units are often used even today for fat-soluble vitamins in pharmacy and medicine. The level of a vitamin in a food is commonly measured in mass units. In the past, the names associated with a disease caused by deficiency of the vitamin were used (e.g. vitamin A was called antixerophthalmic vitamin or the vitamin against night blindness, and vitamin C was known as antiscorbutic vitamin or the vitamin against scurvy). Upper-case letters (vitamin A or vitamin C) were used later. Subsequently it was discovered that several substances have the same physiological effects, and a numeric index on the upper-case letters began to be used (e.g. vitamins A_1 and A_2). Such designations are still commonly in use, although some vitamins have a simple trivial name (e.g. retinol instead of vitamin A_1 and ascorbic acid instead of vitamin C).

The most common method of classifying vitamins is according to their common physical properties, their solubility in water (in a polar environment) and fat (in a non-polar environment). Vitamins are thus divided into two groups:

- **fat-soluble vitamins**, **lipophilic vitamins** (four vitamins)
- **water-soluble vitamins**, **hydrophilic vitamins** (nine vitamins).

Fat-soluble vitamins are vitamin A, D, E and K. Water soluble vitamins include the B group vitamins or vitamin B complex (which refers to all of the known essential water-soluble vitamins except for vitamin C) and vitamin C. The B group vitamins are thiamine, riboflavin, niacin, pyridoxinal derivatives, pantothenic acid, biotin, folacin and the corrinoids.

Some substances referred to as **provitamins** do not show physiological effects themselves but can serve as precursors from which the body can synthesise vitamins. A provitamin of vitamin A_1 (retinol), for example, is β-carotene. The term vitamin sometimes also includes other biologically active substances whose vitamin effect has not been demonstrated reliably or substances needed by other organisms, for example by microorganisms.

The fat-soluble vitamins have different functions. For example, vitamin A_1 (retinol) is required in the production of the visual pigment rhodopsin; its provitamin (β-carotene) likewise acts as a plant pigment and antioxidant.

The function of hydrophilic vitamins is a catalytic effect, since in all organisms they generally occur as cofactors of various enzymes

[1] Other groups of special biocatalysts are hormones that the body is able to synthesise itself. Between these two groups a sharp boundary exists, both groups were earlier called ergons. A hormone is a substance formed in one organ or part of the body and carried in the blood to another organ or part, depending on the specificity of their effects. Vitamin D_3 (cholecalciferol) is often erroneously considered one of the hormones.

The Chemistry of Food, First Edition. Jan Velíšek.
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and play a role in the metabolism of nucleic acids, proteins, carbohydrates, fats and products of secondary metabolism. For example, vitamin B_1 (thiamine) is a cofactor of decarboxylases, dehydrogenases and other enzymes.

The body's need for most vitamins is relatively low. The amounts needed to ensure the normal physiological function of humans is dependent on many factors such as age, sex, health status, lifestyle, eating habits and work-related activity. Many countries have recommendations for the daily intake of vitamins, which are continually revised in accordance with contemporary scientific knowledge and dietary guidelines. Presently, Recommended Daily Allowances (RDAs)[2] are set in the EU (upper number) and United States (lower number) as follows: vitamin A (800/900 μg), vitamin D (5/15 μg), vitamin E (12/15 mg), vitamin K (75/120 μg), thiamine (1.1/1.2 mg), riboflavin (1.4/1.3 mg), niacin (16/16 mg), pantothenic acid (6/5 mg), vitamin B_6 (1.4/1.3 mg), biotin (50/30 μg), folacin (200/400 μg), vitamin B_{12} (2.5/2.4 μg) and vitamin C (80/90 mg).

Water soluble vitamins are generally not stored in the body, or are stored only for a limited time and the excess is excreted in the urine. Lipophilic vitamins are stored mainly in the liver. The **reserve capacity**, defined as the time during which the need for the vitamin is covered by the organism reserves, is the longest for corrinoids (3–5 years) and vitamin A (1–2 years). The reserve capacity for folacin is 3–4 months, for vitamins C, D, E and K, riboflavin, pyridoxine and niacin it is 2–6 weeks, and for thiamine, pantothenic acid and biotin it is only 4–10 days. Reserve capacity is affected by the history of vitamin intake, the metabolic need for the vitamin and the health status of the individual.

The need for vitamins can also be affected by the presence of other food components that can interfere with vitamins in the diet. These substances are called **antivitamins** or **vitamin antagonists**. Antivitamins eliminate the biological effects of vitamins, which can lead to symptoms of deficiency. The activity of antivitamins is based on the following basic principles:

- Structural analogues of vitamins react with the apoenzymes (which act as competitive enzyme inhibitors) or with proteins that transport vitamins (the antivitamin of thiamine is oxythiamine and the antivitamin of retinol is citral, also known as geranial).

- Certain enzymes convert some vitamins into inactive substances (e.g. lipoxygenase indirectly catalyses degradation of vitamin A and its provitamins, thiaminases decompose thiamine).

- Some substances (usually proteins, but also low molecular weight substances) form unusable complexes with vitamins (a typical example is the reaction of biotin with the egg protein avidin or the reaction of the amino acid linatin from flax seeds with pyridoxal).

Commonly used technological processes cannot usually remove antivitamins of the first group, the so-called true antivitamins. The remaining two groups of antivitamins can be largely eliminated by suitable processes or culinary practices (such as heat inactivation of enzymes or denaturation of proteins bound in the non-utilisable protein–vitamin complexes.

A disease resulting from a deficiency of one or more vitamins is **hypovitaminosis** (if vitamin is supplied in insufficient quantity) or **avitaminosis** (complete lack of vitamin manifested by some biochemical processes disorder). Deficiency of vitamins was formerly one of the main causes of many diseases and deaths. Pellagra (deficiency of some B-complex vitamins), scurvy (vitamin C), beriberi (thiamine), rickets (vitamin D), pernicious anaemia associated with reduced ability to absorb vitamin B_{12} (corrinoids) and xerophthalmia (vitamin A) are now well-known diseases caused by vitamin deficiency. Excessive intake of one or more vitamins (especially of lipophilic vitamins A and D) also causes an abnormal state resulting from disturbances of biochemical processes and can lead to severe diseases known as **hypervitaminosis**.

In food, vitamins are found in different amounts, which usually ranging from micrograms per kilogram to hundreds or thousands of milligrams per kilogram according to the particular vitamin, food type and method of processing. Vitamins occur as free compounds and in various bound forms, usually bound to proteins or carbohydrates. Physiological activity generally encompasses more than one entity. For example, the activity of vitamin A has about 50 naturally occurring carotenoids, and vitamin C activity involves two basic compounds, L-ascorbic and L-dehydroascorbic acids.

The most important sources of vitamins are mainly the basic foods, such as meat and meat products, milk and dairy products, eggs (especially egg yolk), bread and other cereal products, fruits and vegetables, which should adequately cover the vitamin requirements. Some foods have high or extremely high vitamin contents (e.g. vitamin C in rosehips), but these are eaten irregularly or rarely and are therefore not a significant source of vitamins for most of the population. Other vitamins are limited to a certain group of foods (e.g. corrinoids are found only in foods of animal origin).

The vitamin contents of foods are affected by a number of factors, in addition to the genetic makeup of the given organism. In foods of animal origin, vitamin content depends mainly on conditions during storage and processing of the raw materials. In foods of plant origin, the vitamin content depends particularly upon climatic conditions during growth, especially rainfall, fertilisation, stage

[2]The recommended daily allowance (RDA) for vitamins (and minerals; see Chapter 6) is defined as the minimum daily amount of nutrient required to avoid a deficiency. The Reference Daily Intake or Recommended Daily Intake (RDI), based on the older Recommended Dietary Allowance (RDA), is the daily intake level of a nutrient that is considered to be sufficient to meet the requirements of 97–98% of healthy individuals. The RDI is still used for nutrition labelling. In 1997, RDA became one part of a broader set of dietary guidelines called the Dietary Reference Intake (DRI) used by both the United States and Canada. The current Dietary Reference Intake recommendation is composed of: Estimated Average Requirements (EAR), expected to satisfy the needs of 50% of the people in that age group; Recommended Dietary Allowances (RDA), the daily dietary intake level of a nutrient considered sufficient to meet the requirements of nearly all (97–98%) healthy individuals in each life-stage and gender group (it is usually approximately 20% higher than the EAR); Adequate Intake (AI), where no RDA has been established; and tolerable Upper intake Levels (UL), to caution against excessive intake of nutrients that can be harmful in large amounts.

of ripeness and post-harvest storage and processing. Vitamins, in general, are very unstable compounds and their loss can be induced by a number of factors. During the food manufacturing and cooking processes and during storage of raw materials and foods, the majority of vitamins undergo losses to a greater or lesser extent. Obviously, losses of vitamins depend on the processing procedure, cooking method, cooking time and temperature. Some vitamins are fairly heat-stable, whereas others are heat-labile. For this reason, vitamins are considered indicators of the use of good manufacturing practices (GMPs), procedures that ensure the quality of the manufacturing processes of products for human consumption. Water-soluble vitamins are mainly leached out into the cooking water and will be lost if this extract is thrown away. The highest losses of fat-soluble vitamins are caused by oxidation. The stability of individual forms of vitamins varies, and depends on external factors and on the specific food and technology used.

In the food industry, vitamins are used to enrich many products, by the processes of restitution and fortification (enrichment). Restitution means the return of the vitamin content to the original level found in the raw material; fortification is enrichment to a higher level needed for physiological or other reasons. Some vitamins have also found use as natural dyes (riboflavin and provitamins A, in particular β-carotene) and as antioxidants (provitamins A, vitamin E and vitamin C). Intake of vitamins currently significantly influences the consumption of various concentrated sources of vitamins such as dietary supplements and multivitamin preparations. Intake of vitamins in these concentrated forms may lead, in extreme cases, to hypervitaminosis.

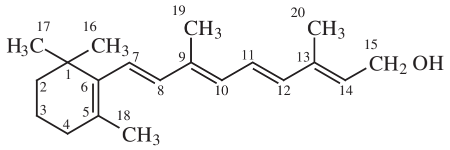
This chapter describes the individual vitamins, their structure, nomenclature, activity in biochemical reactions occurring in the body and their importance in physiology and human nutrition. Their occurrence in foods of animal and vegetable origin and other sources, and their use in food technology, is reported in detail. Another part of the chapter deals with changes in the vitamin contents and their reactions during storage of food materials, and in their culinary and industrial processing. The last part is devoted to biologically active substances that were previously ranked among the vitamins.

5.2 Vitamin A

5.2.1 Structure and terminology

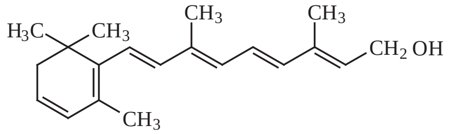
Vitamin A and its provitamins are classified as terpenoids or isoprenoids (see Section 8.2). Provitamins A are tetraterpenes (hydrocarbons) or tetraterpenoids (their oxygen derivatives), which contain 40 carbon atoms in a molecule. They originate, hypothetically, from eight molecules of isoprene (2-methylbuta-1,3-diene). The fission products, known as apocarotenoids, are widespread in living organisms performing many key functions. In animals, apocarotenoids act as vitamins, visual pigments, signalling molecules during cell division, growth and differentiation of tissues and control of reproduction. In plants, apocarotenoids take on the role of hormones, pigments, odorous compounds and perform a series of defensive functions.

The basic and most important biologically active apocarotenoid in animal tissues is all-*trans*-retinol also known as axeroftol or vitamin A_1 (**5-1**). Retinol is an isoprenoid with 20 carbon atoms and five conjugated double bonds in the molecule, more precisely a diterpenic alicyclic alcohol with the so- called β-ionone ring and a side chain of four conjugated double bonds attached to C-6. It is one of 16 possible stereoisomers.



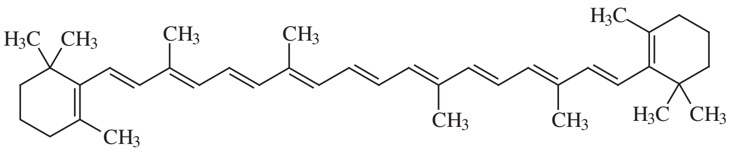
5-1, all-*trans*-retinol

In foods retinol is accompanied by a number of analogues and metabolites, differing in the ionone cycle or side chain structures. Freshwater fish contain, for example, 3,4-didehydroretinol known as vitamin A_2 (**5-2**), which has about 40% of the activity of retinol. Marine fish, birds and mammals do not synthesise this vitamin. Synthetic derivatives related chemically to vitamin A are collectively called **retinoids**. These substances are used for the treatment of various skin conditions, such as severe acne, sun spots, wrinkles and psoriasis. Some retinoids may even help treat or prevent certain forms of skin cancer.

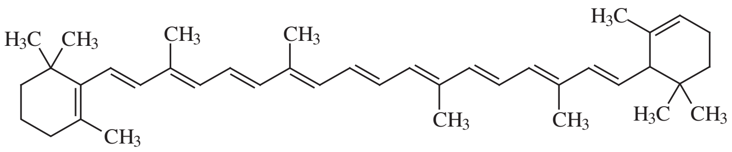


5-2, 3,4-didehydroretinol

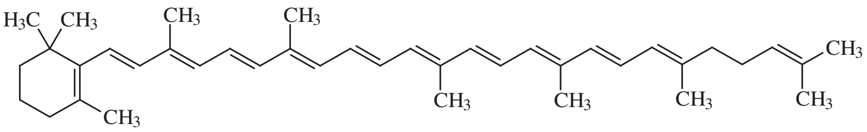
Activity of vitamin A (antixerophthalmic vitamin) have about 50 other naturally occurring compounds from the group of carotenoids, which are called provitamins A. The most important provitamin A is β-carotene (**5-3**). In foods it is often accompanied by other carotenes, namely α-carotene (**5-4**) and γ-carotene (**5-5**) and xanthophylls, such as β-cryptoxanthin (**5-6**) and echinenone (**5-7**) and other provitamins A.



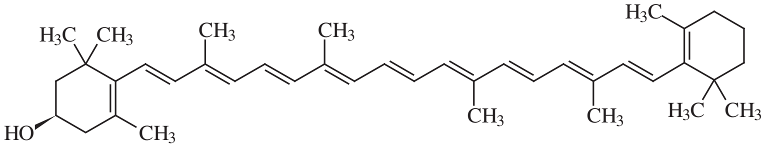
5-3, β-carotene



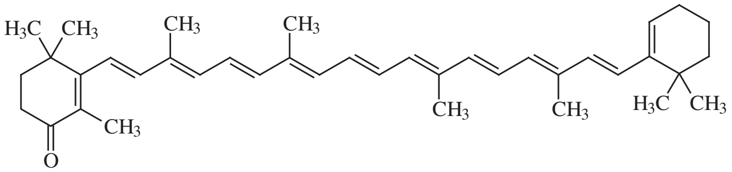
5-4 α-carotene



5-5, γ-carotene



5-6, β-cryptoxanthin

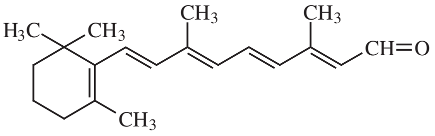


5-7, echinenone

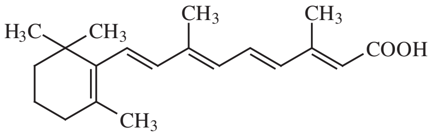
5.2.2 Biochemistry

The building unit of vitamin A and other isoprenoids is not isoprene, but its activated forms isopentenyl diphosphate and dimethylallyl diphosphate. Isopentenyl diphosphate is synthesised either from acetylcoenzyme A (an intermediate of its biosynthesis is mevalonic acid) or, alternatively, from 1-deoxy-D-xylulose 5-phosphate via 2-*C*-methyl-D-erythritol 4-phosphate and isopentenyl diphosphate. Gradual lengthening of the isoprenoid chain yields the immediate precursor of the carotenoids, geranylgeranyl diphosphate, with 20 carbon atoms in the molecule. The subsequent reactions of two molecules of geranylgeranyl diphosphate yield provitamins A (see Section 3.7.4.2).

In animals, birds and fish, provitamins A containing at least one β-ionone ring are transformed largely by central (symmetric) fission of the molecule catalysed by β-carotene-15,15′-dioxygenase to all-*trans*-retinal, also known as retinaldehyde (**5-8**). Two molecules of retinal are formed from one molecule of β-carotene, while other provitamins A provide only one molecule of retinal. Cleavage of β-carotene between the two cyclohexyl rings may also occur on other double bonds. This asymmetric (eccentric) cleavage provides two β-apocarotenals with different chain lengths. Subsequent cleavage of the β-apo-carotenal with the longer chain length gives retinal, but only one molecule per one molecule of β-carotene. Retinal is reversibly reduced to all-*trans*-retinol by retinol dehydrogenase. Together with other biologically active forms of vitamin A, such as all-*trans*-retinoic acid (**5-9**), which is the product of irreversible oxidation of retinal by retinal dehydrogenase, all-*trans*-retinol esters with higher fatty acids (**5-10**), all-*trans*-retinyl β-glucuronide (**5-11**) and other compounds, all-*trans*-retinal is stored mainly in the liver and transported, bound to specific proteins, by plasma. Retinoic acid occurs in a level of about 0.000 1%, in rosehip seed oil (the total oil content in seeds is approximately 9%).

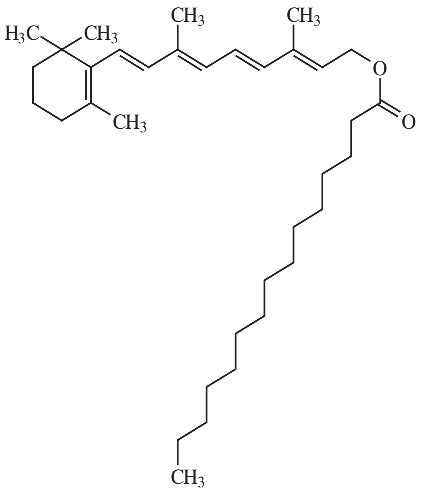


5-8, all-(*E*)-retinal

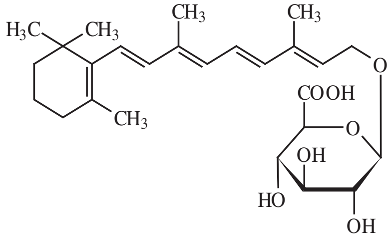


5-9 all-(*E*)-retinoic acid

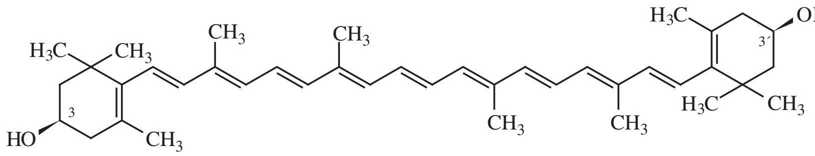
In addition to β-carotene, freshwater fish can also convert the xanthophyl lutein (**5-12**), also known as 3,3′-dihydroxy-α-carotene or (3*R*,3′*S*,6′*R*)-β,ε-carotene-3,3′-diol, into vitamin A. Lutein eliminates one molecule of water (C-3′ hydroxyl group) and yields anhydrolutein (**5-13**), which cleaves to the corresponding aldehydes. These aldehydes are reduced to all-*trans*-3,4-didehydroretinol (vitamin A_2) and all-*trans*-3-hydroxyretinol, respectively. The latter compound can dehydrate to form another



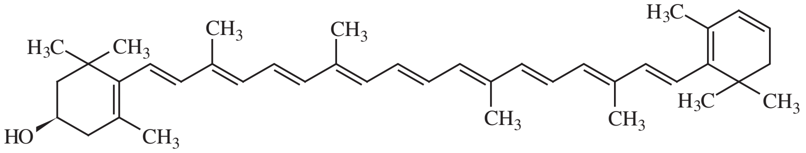
5-10, all-(*E*)-retinyl palmitate



5-11, all-(*E*)-retinyl β-D-glucuronide



5-12, lutein



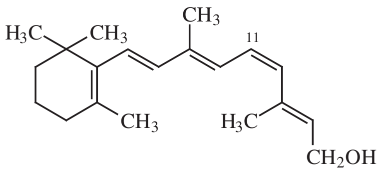
5-13, anhydrolutein

molecule of vitamin A_2. Trivial, specific, semi-systematic and systematic names of some provitamins A are given in Table 5.1.

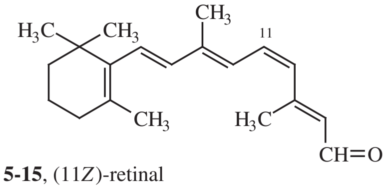
The biochemistry of vision is a very complex process, where all-*trans*-retinol isomerises to 11-*cis*-retinol (**5-14**), which is then enzymatically oxidised to 11-*cis*-retinal (**5-15**). This compound associates with light-sensitive membrane-bound proteins (35–55 kDa) called opsins. Ciliary opsins (*c*-opsins) are typical of vertebrates, while invertebrates usually have rhabdomeric opsins (*r*-opsins). The resulting complexes are visual pigments (photoreceptors), which initiate a cascade that converts light (photons) falling on the retina into neural signals. Light destabilises opsins, which leads to protein conformation changes and isomerisation of 11-*cis*-retinal to all-*trans*-retinal. In order to function, each eye needs, in addition to opsins, the so-called shielding pigment that shields the retina from excess incoming light and exposes the photoreceptors to light coming from only a certain direction, thus ensuring the perception of directional light. Melanin (see Section 9.3.1.1) is a typical shielding pigment of vertebrates; invertebrates mainly have pteridines (see Section 9.3.4) and phenoxazines (see Section 9.3.7) at their disposal. During biological inactivation (catabolism), retinol is oxidised at carbon C-4 to a hydroxyl- or oxoderivative or the side chain can be shortened or hydroxylated at the C-5 methyl group of the β-ionone ring.

Table 5.1 Trivial, specific, semi-systematic, and systematic names of important provitamins A.

Compound
α-Carotene, β,ε-carotene
β-Carotene, β,β-carotene
γ-Carotene, β,ψ-carotene
β-Cryptoxanthin, 3-hydroxy-β,β-carotene
Echinenone, β,β-caroten-4-one
all-*trans*-Retinal, retinal, retinen, vitamin A_1 aldehyde, 15-apo-carotene-15-al, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraenal
all-*trans*-Retinol, retinol, vitamin A_1, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-ol
all-*trans*-Retinoic acid, retinoic acid, tretinoin, vitamin A_1 acid, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-carboxylic acid
all-*trans*-3,4-Didehydroretinol, 3,4-didehydroretinol, vitamin A_2, (2*E*,4*E*,6*E*,8*E*)-3,7-dimethyl-9-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)nona-2,4,6,8-tetraen-1-ol



5-14, (11*Z*)-retinol



5-15, (11*Z*)-retinal

5.2.3 Physiology and nutrition

Absorption of the individual provitamins is not always quantitative. It is dependent on food composition and method of cooking, especially on the presence of lipids and their concentration. For example, the amount of β-carotene needed for the formation of 1 μg of retinol is 4 μg (if β-carotene is present in milk, margarine, vegetable oils or animal fats), 8 μg (in cooked leafy vegetables or carrot cooked in fat) or even 12 μg (if present in carrot boiled in water). The provitamin in raw carrot is almost non-utilisable.

The recommended daily dose of retinol in children is 0.4–0.6 mg and 0.8–1.0 mg in adults. On average it is 0.9 mg or 3000 IU, which is equivalent to 1.8 mg (3000 IU) of β-carotene in vitamin preparations or to 10.8 mg (18 000 IU) of β-carotene in foods. For pregnant and lactating women, the recommended daily dose is 1.0–1.2 and 1.2–2.0 mg, respectively.[3] About 50% of the vitamin A requirement is covered (in some countries for the most part) by provitamins in food of plant origin. About 40% of the required amount is provided by provitamins from vegetables, 20% by retinol and provitamins from meat and meat products, 15% by retinol and provitamins from milk and dairy products, 8% by fruit provitamins, 8% by retinol from fats (vegetable oils contain only provitamins) and 6% by retinol and provitamins from eggs. The biological activity of 9-*cis*-retinol is 21%, of 11-*cis*-retinol 24%, of 13-*cis*-retinol 75%, of 9,13-di-*cis*-retinol 24%, of 11,13-di-*cis*-retinol 15% and of all-*trans*-retinal 90% of the activity of all-*trans*-retinol.

Avitaminosis manifests itself as disturbed vision (night blindness) and keratinisation of mucous membranes (which line the respiratory tract, intestines, urinary tract and epithelium of the eye), the inhibition of growth, and in deformation of bones and reproductive organs. Reduced absorption of vitamin A can lead to hypovitaminosis, for example, in vegans.

High doses of vitamin A result in increased liver reserves of vitamin A and hypervitaminosis symptoms (acute or chronic intoxication with various symptoms including strumigenicity). Some individuals have genetically conditioned susceptibility to retinol, which is manifested by intolerance even at doses only slightly higher than normal doses. Excessive intake of provitamin A β-carotene by vegetarians and children (hypercarotenosis or carotenaemia or xanthaemia) does not produce symptoms of hypervitaminosis (an excessive intake of provitamins from carrots or other vegetables worsens their resorption), but may manifest itself through the presence of β-carotene in plasma and by temporary yellow–orange discolouration of the skin, which dissipates when materials containing β-carotene are eliminated from or reduced in the diet.

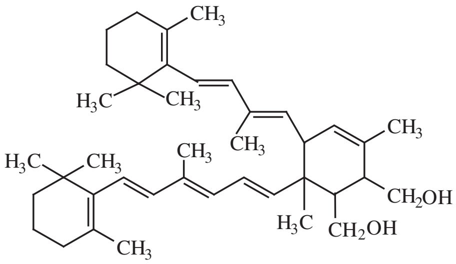
Provitamins A exhibit anticarcinogenic effects, because they are part of the control mechanisms that scavenge free radicals (toxic forms of oxygen). Their antioxidant potential is relatively low. Other carotenoids that do not act as provitamins A have significantly higher antioxidant activities, such as lycopene, zeaxanthin and lutein. The enzymes that oxidise fatty acids (lipoxygenases or linoleate: O_2 oxidoreductases, formerly known as lipoxidases) are antivitamins A.

5.2.4 Use

To enrich foods with vitamin A (e.g. table oils, margarine, butter, dairy products and flour), synthetic and relatively stable retinyl acetate or retinyl palmitate (**5-10**) are used, β-carotene (**5-4**) is used as a lipophilic dye.

5.2.5 Occurrence

Retinol does not occur in foods of plant origin, higher fungi or microorganisms (bacteria, yeasts and moulds), but these materials often contain carotenes and xanthophylls that show the activity of provitamin A. Animals are unable to synthesise carotenoids *de novo*; they only convert plant pigments into substances of a different structure or store them as such (Table 5.2). These reactions generate retinol, 3,4-didehydroretinol and a retinol dimer known as kitol (**5-16**), which can be obtained from whale liver oil in particular. The latter substance has little or no biological activity, but is transformed into retinol upon heating to temperatures above 200 °C. Also, some rosy pink pigments of fish (such as salmon) and some crustaceans (such as shrimps), and meat and feathers



5-16, kitol

[3]The total content of vitamin A is expressed in International Units (IU) and earlier it was in retinol equivalents (RE). Thus 1 IU is equal to 0.3 μg of retinol, 0.6 μg of β-carotene or 1,2 μg of other provitamins A (such as α-carotene, γ-carotene, β-cryptoxanthin and echinenone) and 1 RE is equal to 1 μg of retinol, which is equivalent to 3.33 IU of vitamin activity of retinol or 10 IU of vitamin activity derived from β-carotene. Vitamin A in vitamin preparations is often in the form of more stable retinyl acetate; 1 IU is equivalent to 0.33 μg of retinyl acetate. In animal materials, the amount of vitamin A (expressed in RE) equals the sum of μg of retinol and μg of β-carotene/6 or IU of retinol/3.33 IU + IU of β-carotene/10. In plant materials, it is equal to the sum of μg of β-carotene/6 and μg of other retinoids/12.

Table 5.2 Retinol and provitamins A contents of some foods.

Food	Edible portion (mg/kg or mg/l)		Food	Edible portion (mg/kg or mg/l)	
	Vitamin A	Provitamin A		Vitamin A	Provitamin A
Meat	0.1	0.4	Carrots	-	20-95
Liver	30-400	300	Parsley (root)	-	0.1
Milk	0.3 -1.0	0.1-0.6	Parsley (curly)	-	30-260
Cheeses	1.6-3.2	0.3-8.0	Cabbage	-	3.0-74
Eggs	0.5-1.5	0.1-2.0	Savoy cabbage	-	50
Fish	0.5	0.7	Broccoli	-	25
Butter	5.0-10	4.0-8.0	Cauliflower	-	0.3
Apples	-	0.1-0.3	Lettuce	-	3.0-25
Apricots	-	6.0-20	Spinach	-	50-480
Bananas	-	0.3-2.3	Tomatoes	-	3.0-90
Oranges	-	0.5-4.0	Peppers	-	3.8-24
Melons	-	20	Peas	-	3.0
Mango	-	20	Beans	-	3.0-5.0

of some birds (such as flamingos) are transformed carotenoids (xanthophylls). The main pigment of the fish and crustaceans is astaxanthin (see Section 9.9.2.4.3), feathers of flamingos contain canthaxanthin and astaxanthin as the main components, together with some minor pigments (echinenone, phoenicoxanthin and phoenicopterone, see Section 9.9.3).

5.2.5.1 Foods of animal origin

The main forms of vitamin A include retinol esterified with higher fatty acids and free retinol or retinal. Precursors, or provitamins A, occur in food of animal origin in relatively small quantities. The most common ester of retinol is palmitate, and esters with other fatty acids are also found in variable amounts. For example, the main component in milk is palmitate followed by oleate and stearate. Other fatty acid esters (caprylate, caproate, linoleate, laurate, arachidonate, linolenate, myristate, palmitoleate, pentadecanoate, gadoleate and heptadecanoate) and free retinol are also present in smaller amounts. One particularly rich source of vitamin A is liver. For example, the retinol content in pork liver is about 30 mg/kg, and the liver of polar bears contains up to 60 g/kg of retinol. There is here a clear correlation with their diet, which consists mainly of seals. Milk (as well as meat) contains relatively little vitamin A (the vitamin content is proportional to the fat content). Dairy products and butter are good sources of vitamin A because of their high fat content.

5.2.5.2 Foods of plant origin

The most important provitamin A is β-carotene. Leafy vegetables such as spinach and cabbage are very rich sources of provitamins A as they contain 10–30 mg/kg retinol equivalents (RE), mainly in the form of β-carotene. Provitamins A form about 25% of total carotenoid pigments present in these vegetables. The classic source of β-carotene is carrots, containing about 20 mg/kg RE. The tomato has lycopene as the main pigment, which is not a provitamin A and the amount of β-carotene is relatively low (about 6 mg/kg). Tomatoes also contain small amounts of γ-carotene (1 mg/kg). There is a high content of β-carotene in the orange varieties of tomatoes (Table 5.2). Some fruits (such as apricots and mango) are also good sources of vitamin A precursors. Margarine is usually fortified with synthetic retinyl acetate or palm oil (that contains mainly α-carotene), so that the vitamin content is the same as in butter.

5.2.5.3 Other sources

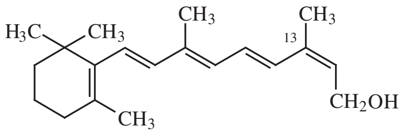
Fish liver oil is a very abundant source of vitamin A. Cod liver oil contains 10–100 g/kg of retinol (or its esters). Liver oils of freshwater fish contain about 40 mg/kg of retinol and 110 mg/kg of 3,4-didehydroretinol, the ratio of these vitamins A is highly variable.

5.2.6 Reactions

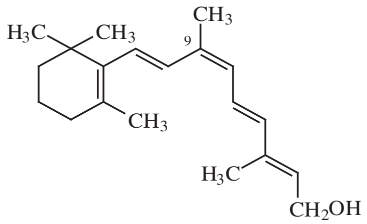
Naturally occurring all-*trans* isomers of provitamin A and retinol are unstable compounds. They isomerise very easily during food storage, especially when exposed to light and higher temperatures (cooking, baking and other thermal operations). They are also sensitive to oxidation (lipoxygenases, oxygen in the air or chemical agents). These compounds likewise react with free radicals and thus inhibit unwanted radical oxidation reactions. Similarly to lipid oxidation, a range of products are formed due to the combined effect of various factors.

5.2.6.1 Retinol

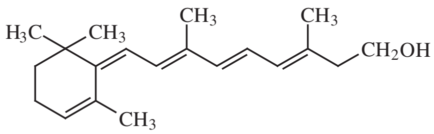
Retinol and its derivatives isomerise to a mixture of products in which 13-*cis*- (**5-17**) and 9-*cis*-stereoisomers (**5-18**) dominate. These isomers of retinol generally have a less intense colour than all-*trans* isomers. At the same time, and especially in acidic media, there is a shift of the double bonds towards the β-ionone ring with the formation of a positional isomer, retrovitamin A, also known as α-retinol (**5-19**). Retrovitamin A partially dehydrates to all-*trans* anhydroretinol (anhydrovitamin A, **5-20**).



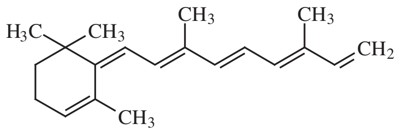
5-17, (13*Z*)-retinol



5-18, (9*Z*)-retinol

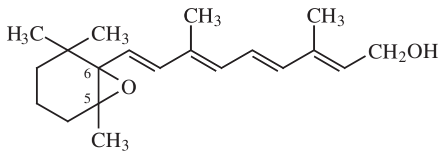


5-19, α-retinol



5-20, all-(*E*)-anhydroretinol

Oxidation of the β-ionone ring of retinol yields unstable hydroperoxides as the primary products. Their subsequent reactions produce relatively stable epoxides, such as all-*trans*-5,6-epoxyretinol (**5-21**), and oxidation of the hydroxymethyl group produces all-*trans*-retinal. Other oxidation products include compounds with shorter side chains. These oxidation products of retinol or the corresponding free radicals yield polyene polymers. Similar reactions *in vivo* lead to the yellow–brown aging pigment lipofuscin (see Section 3.6.1). Retinol esters are more stable to oxidation than free retinol. Tocopherols exhibit protective effects.

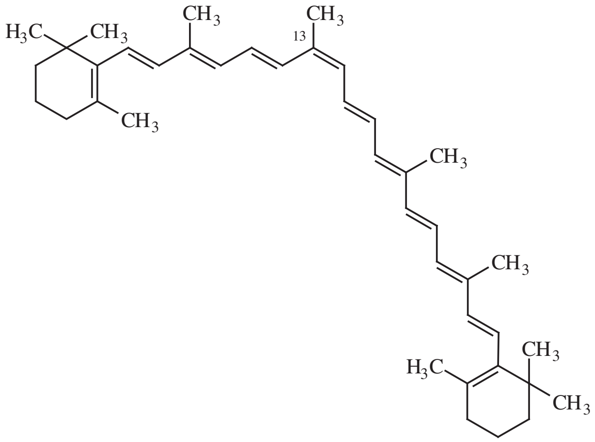


5-21, all-(*E*)-5,6-epoxyretinol

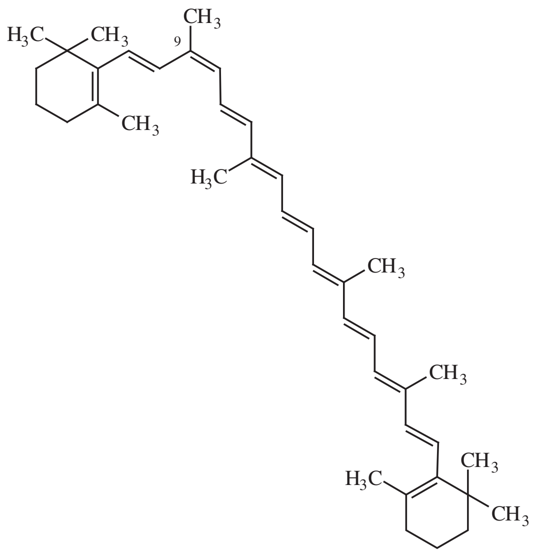
5.2.6.2 β-Carotene

Similarly to retinol, isomerisation, oxidation and degradation of β-carotene and other provitamins A may occur during food storage and processing through the combined effects of light, heat, oxygen, hydroxonium ions and other factors. Some stereoisomers of β-carotene, such as 13-*cis*-β-carotene (**5-22**) and 9-*cis*-β-carotene (**5-23**), appear as minor natural pigments in fruits and vegetables, especially in green species where photosynthesis occurs. This is explained by the presence of chlorophylls that act as photosensitisers and catalyse photoisomerisation of β-carotene to 13-*cis*- and 9-*cis*-isomers.

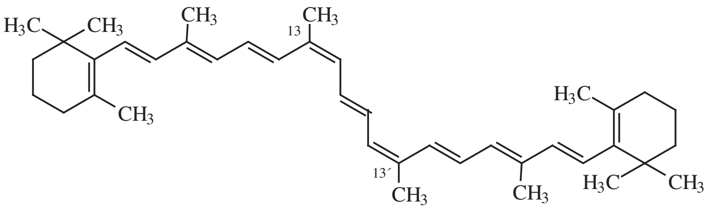
Stereoisomers of β-carotene are also formed during storage of flour under normal conditions and in particular at higher



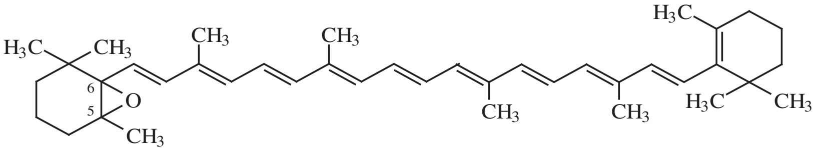
5-22, (13*Z*)-β-carotene



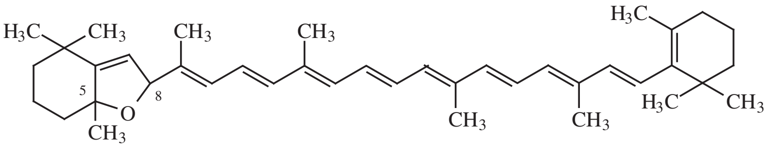
5-23, (9*Z*)-β-carotene



5-24, di-(*Z*)-β-carotene



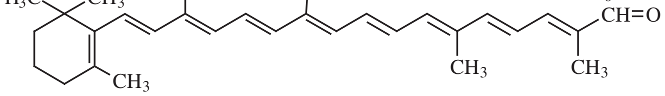
5-25, β-carotene-5,6-epoxide



5-26, β-carotene-5,8-epoxide

temperatures common in cooking, baking, frying and extrusion. Frying of foods or extrusion of cereals (at temperatures of 180–200 °C) yields 13-*cis*-β-carotene (as the major product) and other stereoisomers, such as 9-*cis*-β-carotene, 15-*cis*-β-carotene, 13,13′-di-*cis*-β-carotene (**5-24**), 9,9′-di-*cis*-β-carotene, 9,13′-di-*cis*-β-carotene and other products. Oxidation products, such as 5,6-epoxide (**5-25**), are formed at the same time.

In acidic media, 5,6-epoxides isomerise to dihydrofuran derivatives, also known as 5,8-epoxides (**5-26**), other products include diepoxides, β,β-carotene-3,3′-diol (zeaxanthin), β,β-carotene-4-one (echinenone) (**5-7**) and their degradation products, such as β-apo-8′-carotenal (**5-27**), β-apo-10′-carotenal, β-apo-12′-carotenal, β-apo-14′-carotenal and numerous other aldehydes. The main product is 5,8,5′,8′-diepoxide (also known as 5,8,5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene or aurochrome or ξ-carotene), and other products include 5,6,5′,6′-diepoxide (5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene) and 5,6,5′,8′-diepoxide (5,6,5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene or luteochrome).



5-27, β-apo-8′-carotenal

5.2.6.2.1 Reactions with free radicals

Carotenoids react with free radicals, they deactivate them and thus act as antioxidants. They exhibit antioxidant effects in proportion to their concentration in systems containing lipids and also *in vivo*. The mechanism of the antioxidant action of carotenoids differs from the mechanism of action of vitamin E or synthetic phenolic antioxidants. In heterogeneous systems, such as emulsions, there are no significant differences between individual carotenoids. In homogeneous systems, such as anhydrous fats and oils, individual carotenoids differ somewhat in their antioxidant properties.

It is assumed that the hydroperoxyl radical ROO• generated by autoxidation of lipids is not reduced to hydroperoxide as in the case of phenolic antioxidants, but is captured by the conjugated polyene system, represented by the formula **5-28**, with the formation of relatively stable β-carotene radicals, these being stabilised by resonance:

$$\text{R–O–O}^\bullet + \beta\text{-carotene} \rightarrow \text{R–O–O–}\beta\text{-carotene}^\bullet$$

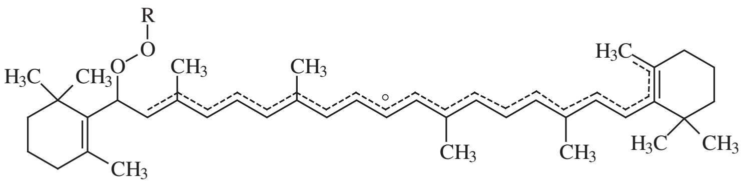
These radicals break down to alkoxyl radicals (RO•) and stabilise through the formation of epoxides, carbonyl compounds and a number of other products (Figure 5.1):

$$\text{R–O–O–}\beta\text{-carotene}^\bullet \rightarrow \text{R–O}^\bullet + \beta\text{-carotene epoxide}$$
$$\beta\text{-carotene epoxide} \rightarrow \text{polar products}$$

The main products of the reaction outlined in Figure 5.1 are 5,6- and 5,8-epoxides, 19-oxomethyl-10-nor-β,β-carotene, 12-formyl-11-nor-β,β-carotene, its isomer 13,15′-epoxyvinyleno-13,15′-dihydro-β,β-carotene and 13,14-epoxide that is transformed into 11,15′-cyclo-12,15-epoxy-11,12,15,15′-tetrahydro-β, β-carotene.

Under anaerobic conditions and in the presence of small amounts of oxygen, when carotenoids show higher antioxidant activity, β-carotene reacts with another hydroperoxyl radical with the formation of stable polar products:

$$\text{R–O–O–}\beta\text{-carotene}^\bullet + \text{R–O–O}^\bullet \rightarrow \text{polar products}$$



5-28, resonance stabilised R–O–O–β-carotene radical

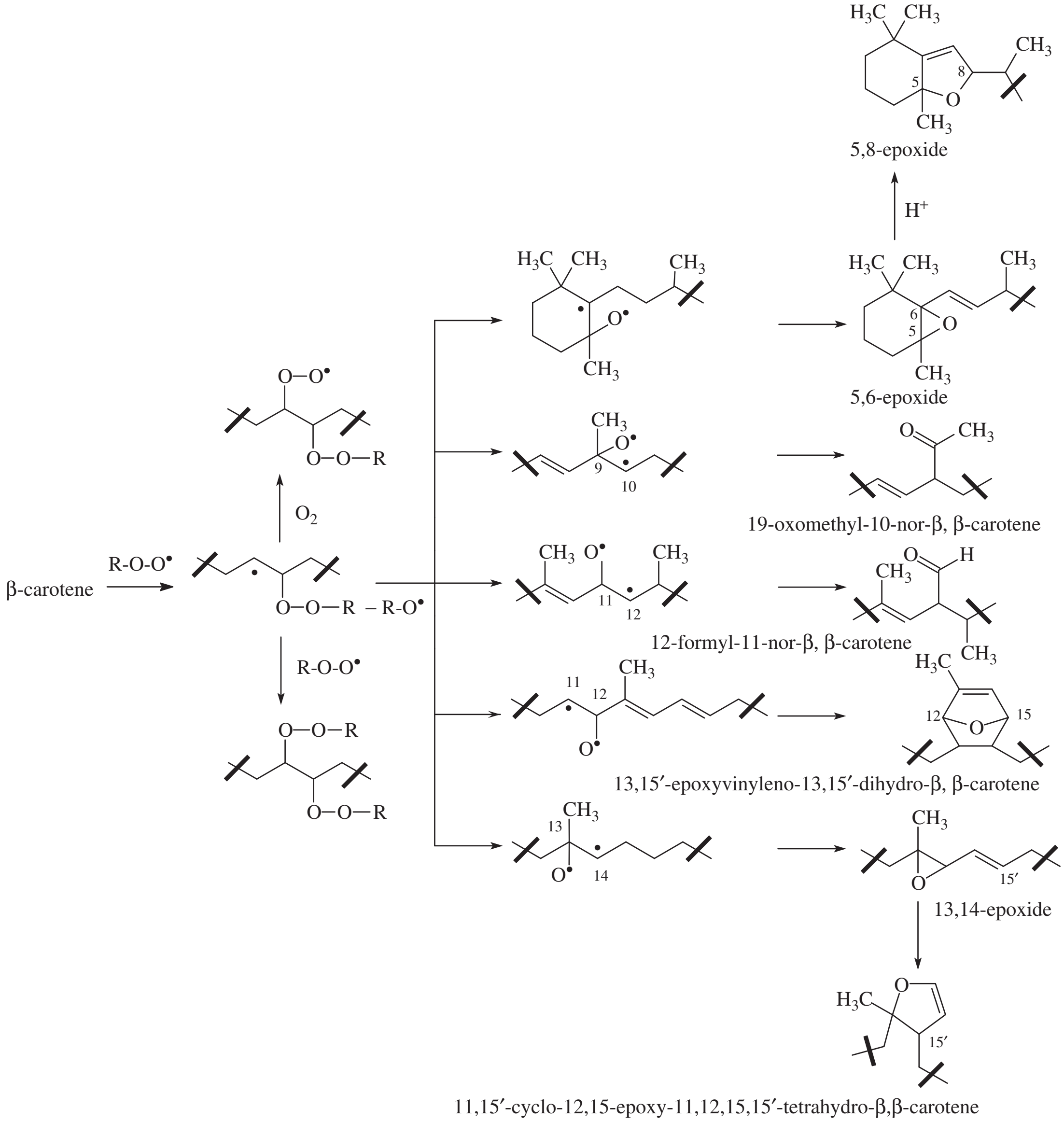


Figure 5.1 Reaction of β-carotene with lipid hydroperoxyl radicals (R = lipid residue).

At higher partial pressure of oxygen (e.g. during storage of fat in contact with air) unstable peroxyl radicals can form:

$$\text{R–O–O–}\beta\text{-carotene}^\bullet + O_2 \rightarrow \text{R–O–O–}\beta\text{-carotene–O–O}^\bullet$$

Alternatively, stable polar products are formed:

$$\text{R–O–O–}\beta\text{-carotene–O–O}^\bullet \rightarrow \text{polar products and radicals}$$

In reactions involving the hydroperoxyl radical R–O–O$^\bullet$, the alkoxyl radical RO$^\bullet$ formed by decomposition of hydroperoxides can also react with β-carotene.

β-Carotene also deactivates singlet oxygen ($^1O_2$; see Section 3.8.1.8.4). In other words, β-carotene acts as a singlet oxygen quencher and prevents lipid oxidation initiated by singlet oxygen. Singlet oxygen is usually generated by the interaction of air oxygen

(triplet oxygen, $^3O_2$) with various photosensitisers. The reaction products are oxygen in the triplet state and β-carotene in the excited triplet state (^{3}β-carotene*):

$$^1O_2 + \beta\text{-carotene} \rightarrow {}^3O_2 + {}^3\beta\text{-carotene}^*$$

β-Carotene is more effective in the presence of tocopherols, which ensure its protection against oxidation. Degradation of β-carotene and other carotenoids produces a series of low molecular weight products such as various hydrocarbons and oxygen compounds (e.g. epoxides and ketones), which are important flavour constituents of many foods.

5.2.7 Changes in foods

Natural provitamins A and vitamin A, β-carotene in foods of plant origin as well as esters of retinol in foods of animal origin, are relatively stable substances in the absence of air. At higher temperatures and in the light (e.g. during food preservation), they may isomerise to **neocarotenes**, which still exhibit vitamin A activity if they have preserved at least one β-ionone ring, but are less intensely coloured. Autoxidation (oxidation that occurs in the open air or in the presence of oxygen) of these substances is particularly rapid in dehydrated food. Retinoids also react with lipid oxidation products (fatty acid oxidation products), which similarly generate less coloured products.

5.2.7.1 Meat and meat products

During normal processing of meat and offal, vitamin A and provitamins A are very stable.

5.2.7.2 Milk and dairy products

During pasteurisation, in UHT milk and during milk drying, up to 6% of vitamin A is lost, and further losses occur during storage. In UHT milk, these losses are 3–7% over 4 weeks. In the presence of oxygen and in light (during storage in inappropriate containers), however, vitamin losses may reach up to 20–30% per hour. Dried milk powder is very stable and vitamin losses, even during prolonged storage, do not exceed 10%. The vitamin content in cheeses is higher than in milk (up to 50% or more, according to the fat content). Storage of butter results in only small losses of carotenoids and retinol.

5.2.7.3 Cereals and cereal products

The changes to the provitamins A content of grains are negligible. During storage of flour, provitamins A react with fatty acid hydroperoxides produced by the action of lipoxygenases. During dough mixing and proofing, hydroperoxides oxidise carotenoids and the range of reactions depends on many factors, such as water content. The result is a desirable lighter colour of the final product (e.g. of bread). On the other hand, the loss of carotenoids (up to 75%) is undesirable in the production of pasta. The addition of ascorbic acid, which inhibits lipoxygenase, has a colour stabilising effect. Losses, often higher than 9%, happen in the production of extruded cereal products. The so-called bleaching of flour is also done by chemical oxidising agents such as halogenides and peroxides (see Section 11.4.2.2.1).

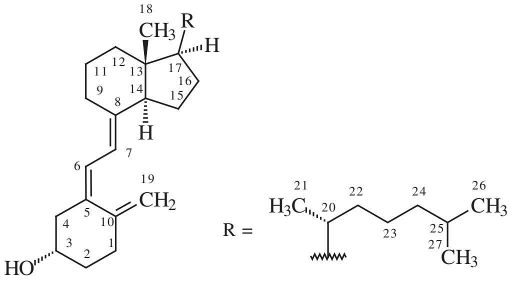
5.2.7.4 Fruits and vegetables

The extent of degradation reactions of carotenoid compounds in preserved fruits and vegetables after blanching and deaeration (removal of air) is usually small. For example, the retention of carotenoids in canned apricots, peaches and plums ranges from 85 to 100% after one year of storage, depending on the type of fruit and storage temperature. In the presence of oxygen a number of isomerisation and fission products of carotenoids arise. Carotenoid substances in dried fruits and vegetables are easily oxidised (the extent of oxidation depends, for example, on water activity, temperature and oxygen content in the atmosphere). During storage of dried carrots in air, there may be up to 50% loss of the carotenoids. There are also extensive losses of carotenoids in the production of fruit wines and spirits. Decomposition of carotenoids, however, also leads to a series of flavour active compounds that can have an impact on odour and taste of the product.

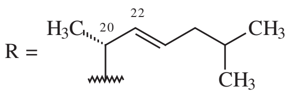
5.3 Vitamin D

5.3.1 Structure and terminology

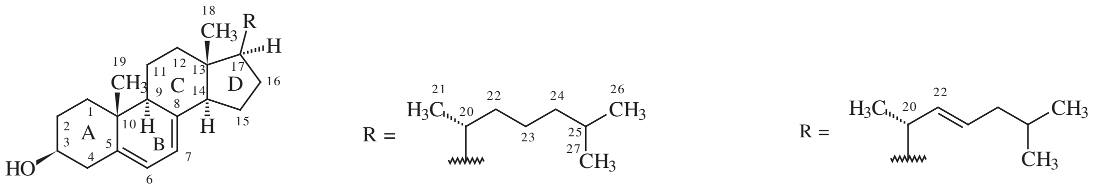
Vitamin D is the common name for a group of closely related lipophilic 9,10-secosteroids of which the most important are vitamin D_3, which is known as cholecalciferol (9,10-seco-$\Delta^{10(19),5,7}$-dehydrocholestatriene-3 β-ol, **5-29**) and vitamin D_2, known as ergocalciferol (9,10-seco-$\Delta^{10(19),5,7,22}$-ergostatetraene-3β-ol, **5-30**).



5-29, cholecalciferol (vitamin D_3)



5-30, ergocalciferol (vitamin D_2)



5-31, 7-dehydrocholecalciferol (provitamin D_3) **5-32**, ergosterol (provitamin D_2)

Vitamins D are produced by ultraviolet (UV) irradiation of their precursors, provitamins D. Provitamins D are cyclopentaperhydrophenanthrenes with C-18 and C-19 methyl groups, C-3 hydroxyl group and a C-5(6), C-7(8) system of conjugated double bonds in ring B, which differ from each other in length and arrangement of the side chain at position C-17. Provitamin D_3 is 7-dehydrocholesterol, also known as 7-procholesterol (**5-31**), provitamin D_2 is ergosterol (**5-32**). In the past, vitamin D_1 was the name for a mixture of vitamin D_2 with lumisterol, a byproduct resulting from irradiation (photodegradation) of ergosterol (Table 5.3). Vitamin D_4 is 22-dihydroergocalciferol, which is produced by irradiation of 22-dihydroergosterol. Irradiation of 7-dehydrositosterol yields sitocalciferol, which is also known as vitamin D_5.

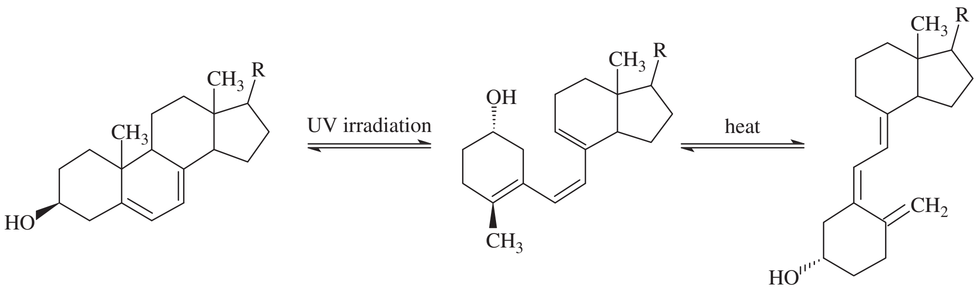
5.3.2 Biochemistry

Provitamins D, similarly to provitamins A, are terpenoids. Gradual lengthening of the isoprenoid chain gives an immediate C_{15} precursor of some steroids, farnesyl diphosphate. Condensation of two molecules of farnesyl diphosphate and subsequent reactions yield provitamins D and other steroids. Through a sequence of reactions, humans and other animals synthesise 7-dehydrocholesterol, which is one of the precursors in the biosynthesis of cholesterol, from lanosterol. UV irradiation of 7-dehydrocholesterol (provitamin D_3) present in the skin cells,[4] at the wavelength range 280–320 nm (with a maximum at 295–297 nm), brings about a photochemical reaction (due to energy absorption by the system of π-electrons), which yields, as the first intermediate, the so-called precholecalciferol (previtamin D_3) with an opened ring B, which spontaneously isomerises, with the migration of hydrogen, to cholecalciferol (Figure 5.2). Precholecalciferol shows about 35% of the activity of cholecalciferol.

[4]Electromagnetic radiation is classified into ultraviolet A radiation (in short UV-A) with a wavelength of 315–400 nm, ultraviolet B radiation (UV-B, 315–280 nm) and ultraviolet C (germicidal) radiation (UV-C, 280–100 nm).

Table 5.3 Nomenclature of major vitamins D and of their precursors.

Trivial name	Systematic name
7-Dehydrocholesterol	7-Dehydrocholest-5-en-3β-ol
(*Z*)-Tacalciol, precholecalciferol, previtamin D_3	(*Z*)-9,10-Secocholesta-5(10),6,8-trien-3β-ol
Cholecalciferol, calciol, vitamin D_3	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β-ol
25-Hydroxycholecalciferol, calcidiol	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β,25-diol
1α,25-Dihydroxycholecalciferol, calcitriol	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-1,3β,25-triol
(*R*)-1,24-Dihydroxycholecalciferol, (*R*)-24-hydroxycalcidiol	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-1,3β,24-triol
(*R*)-1,24,25-Trihydroxycholecalciferol, calcitetrol	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-1,3β,24,25-tetrol
Lumisterol	(*E*)-9β,10α-Ergosta-5,7,22-trien-3β-ol
Ergosterol	(*E*)-Ergosta-5,7,22-trien-3β-ol
Ergocalciferol, ercalciol, vitamin D_2	(5*Z*,7*E*,22*E*)-9,10-Secoergosta-5,7,10(19),22-tetraen-3β-ol



provitamin D previtamin D vitamin D

Figure 5.2 Formation of vitamins D from provitamins D.

Cholecalciferol binds to a specific globulin in blood plasma (vitamin D-binding protein, DBP) and is transported to the liver. Therefore, its concentration in blood plasma is relatively low (<20 nmol/l). In the liver it is stored and, according to requirements, is oxidised to 25-hydroxycholecalciferol, known as calcidiol (Figure 5.3), which is the main circulating metabolite of cholecalciferol. Its concentration in plasma depends on many factors such as the duration of exposure to sunlight and the season. Cholecalciferol is metabolised in the kidneys to a number of dihydroxysubstituted vitamins D_3. The major metabolites are the hormones 1α,25-dihydroxycholecalciferol (known as calcitriol) and (24*R*)-24,25-dihydroxycholecalciferol. The biological efficiency of 1α,25-dihydroxycholecalciferol is about ten times higher than that of cholecalciferol. Along with two other hormones, calcitonin and parathyroid hormone (parathormone, parathyrin), act in the metabolism of calcium and phosphorus. Synthesis of 24,25-dihydroxycholecalciferol is part of a detoxification mechanism, (24*R*)-1,24,25-trihydroxycholecalciferol is a starting compound for catabolism (so-called 24-oxidation) in the target cells, where calcitroic acid is formed as a product. Many other metabolites are known, such as (*S*)-1,25,26-trihydroxycholecalciferol and 1α,25-dihydroxycholecalciferol-26-lactone.

Because of the possibility of its biosynthesis in the body and its biological function (it is an antirachitic vitamin connected with the metabolism of calcium and phosphorus, which are necessary for growth, development and maintenance of bone structure), some reports state that vitamin D_3 is more a hormone than a vitamin, but these statements are wrong and do not reflect the definitions of either a vitamin or a hormone. Cholecalciferol is a vitamin in the true sense of the word, and its metabolite 25-hydroxycholecalciferol (or other deltanoids) is appropriately described as a prehormone. The hormonally active form of vitamin D_3 (hormone) is 1α,25-dihydroxycholecalciferol. Prehormone is a glandular secretory product, having little or no inherent biological potency that is converted peripherally into an active hormone (a prohormone is an intraglandular precursor of a hormone). According to recent research, vitamin D acts not only in the metabolism of calcium and phosphorus, but also in cell differentiation, and plays an important part in the immune system.

Cholecalciferol, ergocalciferol and metabolites of these vitamins ingested via foods are resorbed in the intestines and, bound to DBP, are transported to the liver by the lymphatic vascular system, where they are stored or metabolised.

Commonly used and recommended trivial and systematic names of key vitamins D and related steroids are listed in Table 5.3.

5.3.3 Physiology and nutrition

The vitamin D content of foods is still often given in international units (IU). One IU corresponds to the 0.025 μg of vitamin D_3 or vitamin D_2. Both vitamins have the same biological activity. The most important form of vitamin D is cholecalciferol. The recommended daily dose of vitamin D, which is 2.5–10 μg (there is greater need for the upper limit in babies, children, pregnant

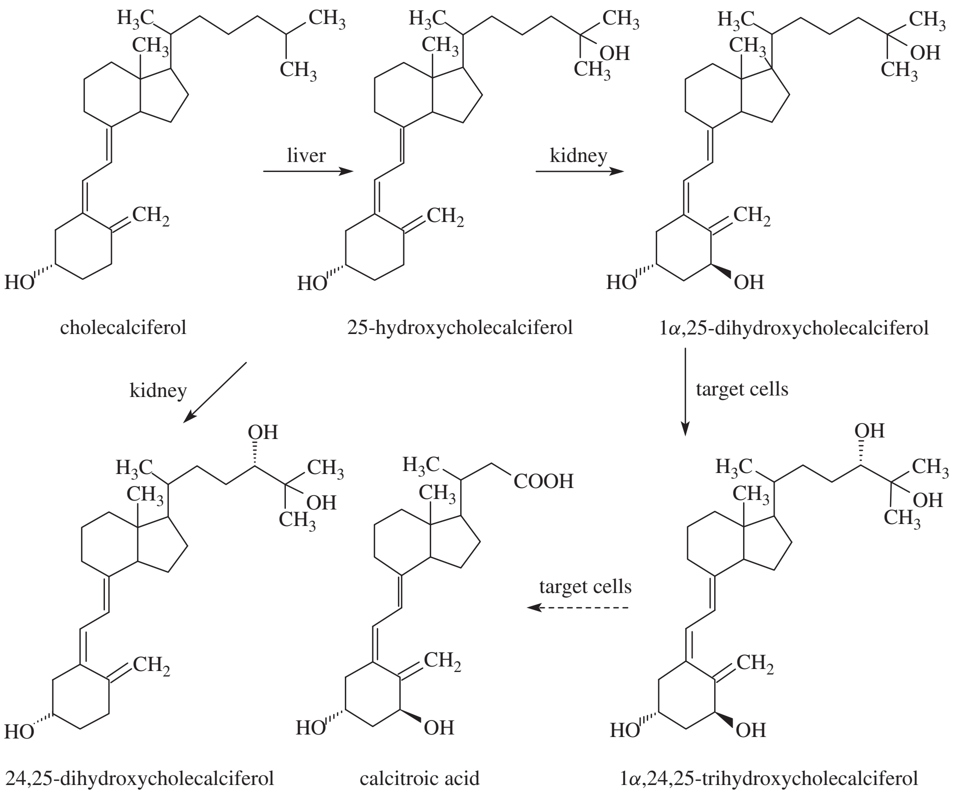


Figure 5.3 Metabolism of cholecalciferol.

and lactating women) is accounted for by the vitamin D_3 obtained through biosynthesis from provitamin 7-dehydrocholesterol in conjunction with the vitamin D_3 and vitamin D_2 contained in foods.

Hypovitaminosis manifests itself as rickets (changes to the skeleton) in children, and in adults by softening and deformation of developed bones caused by defective mineralisation (osteomalacia). Doses higher than the daily requirement similarly negatively result in a variety of symptoms. Long-term high doses of vitamin D may even cause hypercalcaemia, which is due to excessive skeletal calcium release and is characterised by its elevated level in the blood. Calcium is then stored in various organs, for example, in the heart and lungs.

5.3.4 Use

A common practice in some countries is the fortification of margarines by ergocalciferol or dehydrocholecalciferol, and milk and breakfast cereals are likewise often fortified. Ergocalciferol is the main form of vitamin D found in the fortified foods and pharmaceutical preparations. It is produced industrially by photoisomerisation of ergosterol. Some of the earlier procedures of feed enrichment (e.g. yeast for feed purposes) by ergocalciferol were based on irradiation of materials rich in ergosterol.

5.3.5 Occurrence

5.3.5.1 Foods of animal origin

Mammals, birds and fish synthesise cholecalciferol in the same way as man. Cholecalciferol is therefore found naturally in foods of animal origin, where it is also accompanied by its precursor (7-dehydrocholesterol) and related metabolites, such as calcidiol. Notable concentrations of cholecalciferol are found in the liver fats of marine fish (halibut, 30 mg/kg; mackerel, 15 mg/kg; cod, 2.5 mg/kg). Another valuable source is the meat of fatty fish (such as herring, mackerel and salmon).

Lower amounts of vitamin D_3 (cholecalciferol and metabolites) are present in the meat and offal of livestock and in other animal products, such as milk, dairy products and eggs (Table 5.4). Vitamin levels in these foods are, of course, determined by many factors. The cholecalciferol content of milk in the winter, for example, is about four times lower than in the summer.

Table 5.4 Vitamin D content of some foods of animal origin.

Food	Edible portion (μg/kg or μg/l)	Food	Edible portion (μg/kg or μg/l)
Meat	3	Butter	10–20
Liver	2–11	Cheeses	8
Milk	1	Eggs	30–50
Cream	4	Sea fish	50–450

Ergosterol (provitamin D_2) is the main sterol of most fungi, and therefore is naturally present (together with small amounts of vitamin D_2, vitamin D_3 and its metabolites) in cheeses that have molds on the rind or throughout (e.g. blue cheeses such as Roquefort and Gorgonzola).

5.3.5.2 Foods of plant origin

7-Dehydrocholesterol, cholecalciferol, calcitriol and calcidiol can be found in some plant species of the Solanaceae family (also known as the nightshade or potato family) originating in South America. These plants (such a *Solanum glaucophyllum*, syn. *S. malacoxylon*) are calcinogenic plants responsible for producing the enzootic calcinosis of cattle and sheep in Argentina, Brazil, Paraguay and Uruguay. The disease is characterised by the calcification of soft tissues, especially the aorta, heart, lungs and kidneys.

The presence of ergosterol in oil seeds, grains and cereal products (up to hundredths of micrograms per kilogram) is an indicator of microbial contamination. Similarly, ergosterol can be present for the same reason in some vegetables. The content in carrots is reportedly about 0.7 μg/kg, and in cabbage and spinach is about 0.1 μg/kg. Ergosterol is also the main sterol of the plant parasitic fungus ergot (*Claviceps purpurea*), commonly found on grains of rye or sometimes on other grasses such as quack grass (*Elytrigia repens*), and causing a devastating and sometimes deadly syndrome called ergotism in humans and other animals through consumption of contaminated foods and feeds (see Section 12.3.1.2.1).

5.3.5.3 Other sources

The content of ergosterol in the species and strains of yeast *Saccharomyces cerevisiae* (active dried yeast, a type of yeast used to make dough rise in baking) varies within wide limits from 600 to 1500 μg/kg (dry matter).

An important source of ergocalciferol may be higher fungi (of the phylum Basidiomycota that covers most of fungi often referred to as mushrooms), which also contain the provitamin D_2 ergosterol. Ergocalciferol is produced from ergosterol by direct solar radiation with the same mechanism that yields cholecalciferol from 7-dehydrocholesterol (via the corresponding previtamin) in humans. Therefore, mushrooms are the only non-animal-based food containing vitamin D and are hence the only natural vitamin D sources for vegetarians. Cultivated shiitake mushrooms (*Lentinula edodes*) and common mushrooms, white button mushroom (*Agaricus bisporus*), however, have a significantly lower content of ergocalciferol (about 2–3 μg/kg) than wild mushrooms (chanterelle, *Cantharellus cibarius*, around 130 μg/kg, and penny bun, *Boletus edulis*, about 30 μg/kg fresh weight). Generally, the content of ergocalciferol in wild mushrooms depends on many factors, such as climatic conditions and colour of the stalk. Ergocalciferol represents about 90% of the vitamin content, with the remainder being previtamin D_2, 25-hydroxyergocalciferol, ergosta-5,7-dienol (22,23-dihydroergosterol) and ergosta-7-enol. The content of provitamin D_2 (ergosterol) in fungi varies considerably. For example, its content in white button mushrooms is

560 mg/kg, in shiitake mushrooms 849 mg/kg, in oyster mushrooms (*Pleurotus ostreatus*) 680 mg/kg and in chanterelle mushrooms 463 mg/kg fresh weight. Ergosterol usually represents 60–70% of the sterols present.

The concentration of vitamin D_2 in cultivated mushrooms increases if they are exposed to sunlight or artificial UV-B (315–280 nm) or UV-C (280–100 nm) light. After exposure to a UV-B light, the concentration of vitamin D_2 in shiitake mushrooms was increased to 36.7, 68.6 and 106 mg/kg for the pileus, middle, and gill parts, respectively. The concentrations of vitamin D_2 produced in white button mushrooms after exposure to UV-C irradiation were 40.6–141 mg/kg, depending on irradiation doses and time of irradiation.

5.3.6 Reactions

Compounds belonging to the vitamin D group are oxylabile, similar to other lipophilic vitamins. It would therefore be expected that these compounds yield autoxidation products. Thermal transformation (at temperatures around 200 °C) produces both pyroisomers and isopyroisomers of vitamins D (Figure 5.4), isomerisation in acidic media provides isovitamins D and isotachysterols (Figure 5.5).

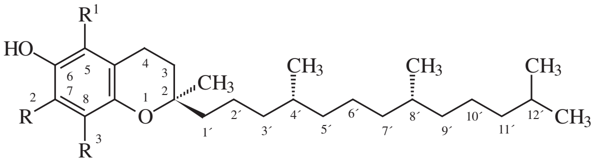
5.3.7 Changes in foods

Some vitamin D reactions, such as formation of oxidation products and isomers, can also be expected in foods. The irradiation of foods may produce photodegradation products that also occur during the industrial production of ergocalciferol from ergosterol. The most important product of ergosterol irradiation is previtamin D_2, but tachysterol, lumisterol and other products are formed as byproducts (Figure 5.6).

5.4 Vitamin E

5.4.1 Structure and terminology

Vitamin E, formerly also known as antisterile vitamin, has eight basic structurally-related derivatives of chroman-6-ol (2*H*-1-benzopyran-6-ol). Structural bases common to all compounds with the reported activity of vitamin E (so-called vitagens E) are tocol (**5-33**) and tocotrienol (**5-34**), which contain a



5-33, tocopherols

α-tocoferol (5,7,8-trimethyltocol), $R^1 = R^2 = R^3 = CH_3$
β-tocoferol (5,8-dimethyltocol), $R^1 = R^3 = CH_3$, $R^2 = H$
γ-tocoferol (7,8-dimethyltocol), $R^1 = H$, $R^2 = R^3 = CH_3$
δ-tocoferol (8-methyltocol), $R^1 = H$, $R^2 = H$, $R^3 = CH_3$
tocol, $R^1 = R^2 = R^3 = H$

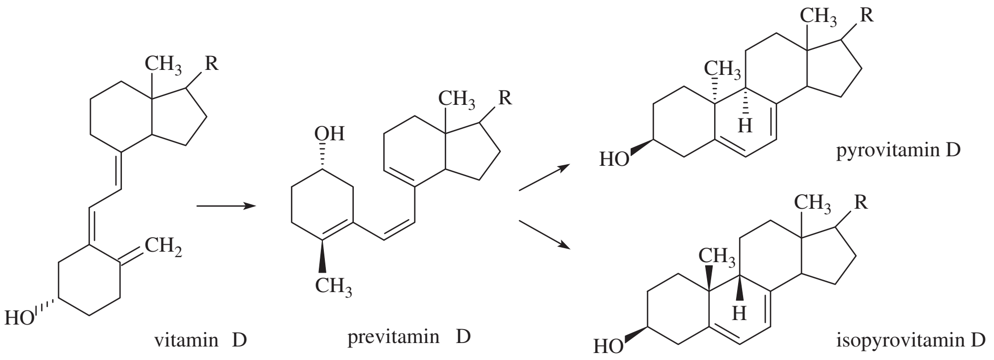


Figure 5.4 Thermal tranformation of vitamins D.

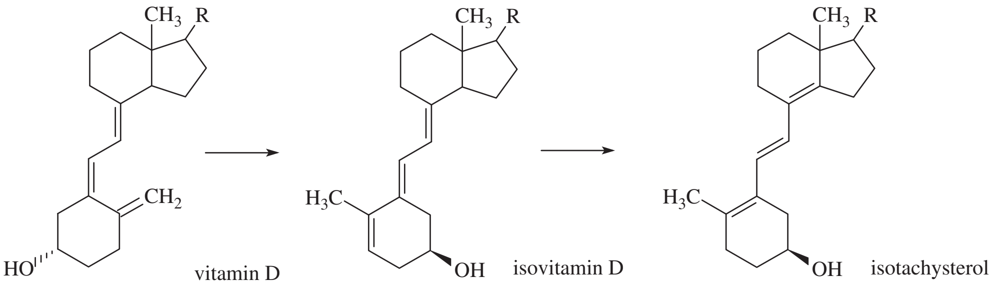


Figure 5.5 Isomerisation of vitamin D catalysed by acids.

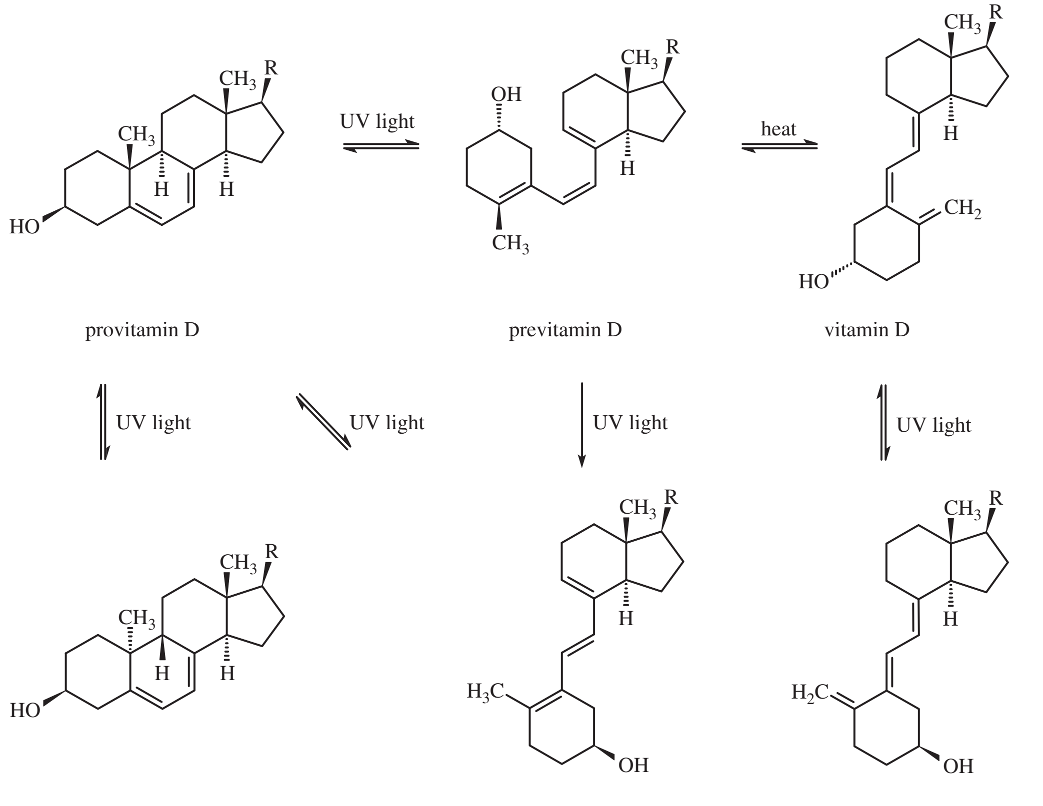
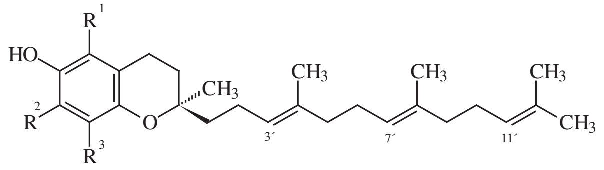


Figure 5.6 Photolytic products of vitamins D.



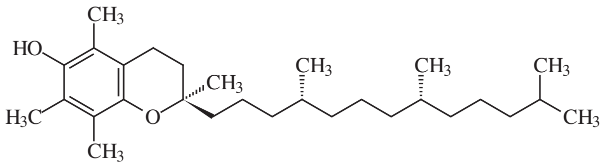
5-34, tocotrienols

α-tocotrienol (5,7,8-trimethyltocotrienol), $R^1 = R^2 = R^3 = CH_3$
β-tocotrienol (5,8-dimethyltocotrienol), $R^1 = R^3 = CH_3$, $R^2 = H$
γ-tocotrienol (7,8-dimethyltocotrienol), $R^1 = H$, $R^2 = R^3 = CH_3$
δ-tocotrienol (8-methyltocotrienol), $R^1 = H$, $R^2 = H$, $R^3 = CH_3$
tocotrienol, $R^1 = R^2 = R^3 = H$

hydrophobic chromane ring with a saturated or unsaturated isoprenoid side chain of 16 carbon atoms. The systematic name of tocol is (2*R*,4′*R*,8′*R*)-3.4-dihydro-2-methyl-2-(4′,8′,12′-trimethyltridecyl)-2*H*-1-benzopyran-6-ol and for tocotrienol is (2*R*,3′*E*,7′*E*,4′*R*,8′*R*,11′*E*)-3,4-dihydro-2-methyl-2-(4′,8′,12′-trimethyltrideka-3′,7′,11′-trienyl)-2*H*-1-benzopyran-6-ol.

The chromane ring is derived from the diterpenic alcohol phytol, with the systematic name (2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol, but in addition to the hydroxyl groups in position C-6 it contains another methyl group at position C-2. The presence of these functional groups is essential for the biological activity of all vitamers. Four forms of vitamin E with a saturated terpenoid side chain derived from the tocol are called **tocopherols** (**5-33**); four forms with unsaturated side chains derived from tocotrienol are called **tocotrienols** (**5-34**). Tocopherols and tocotrienols are collectively known as **tocochromanols**. By virtue of the phenolic hydrogen on the 2*H*-1-benzopyran-6-ol nucleus, the individual compounds exhibit varying degrees of antioxidant activity, depending on the site and number of methyl groups and the type of isoprenoid side chain. The structurally related, but biologically inactive, 5,7-dimethyltocol was formerly called ζ-tocopherol, 5-methyltocol was ε-tocopherol and 7-methyltocol was η-tocopherol.

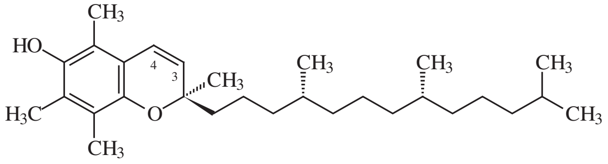
Owing to the presence of three chiral centres (position C-2 of the chromane ring and position C-4′ and C-8′ in the side chain of tocol), each tocopherol can exist in eight diastereoisomeric forms. In nature, however, only (2*R*,4′*R*,8′*R*)-isomers of tocopherols exist. They are also known as (*RRR*)-isomers, 2ᴅ, 4′ᴅ,8′ᴅ-tocopherols, d-tocopherols and (+)-tocopherols. One representative is, for example, (*RRR*)-α-tocopherol, abbreviated to α-T (**5-35**). Tocotrienols containing three double bonds in the side



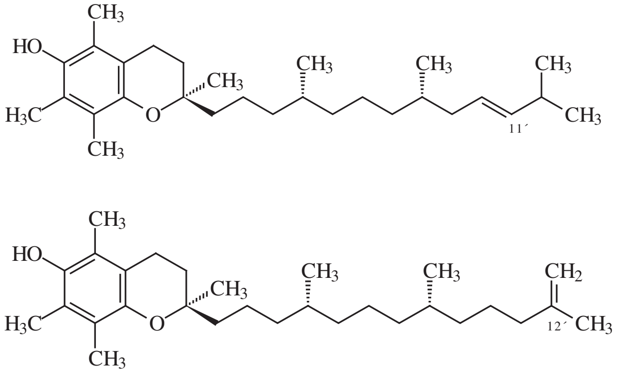
5-35, (2*R*,4′*R*,8′*R*)-α-tocopherol

chain may occur in eight different *Z* (*cis*) and *E* (*trans*) isomers and their combinations. In nature only all-*trans* geometric isomers occur. α-Tocotrienol is often called α-T-3 or α-TT for short.

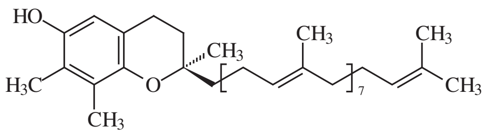
Some vegetable oils also contain small amounts of 3,4-dehydrotocopherols (3,4-dehydrochromenols, **5-36**) and analogues of tocotrienols, tocomonenols and tocodienols that have a partially saturated terpenoid side chain. α-Tocomonoenol (**5-37**) occurs, for example, in crude palm oil (40 mg/kg), with α-tocomonoenol (17.6 mg/kg) and γ-tocomonoenol (118.7 mg/kg) being found in roasted pumpkin seed oil and δ-tocomonoenol was identified in the freeze-dried peel and pulp of kiwi fruits. α-Tocomonoenol, denoted by α-T_1, is similar to α-tocopherol, but with a double bond at the side chain. There are only two known naturally occurring isomers. One with a double bond at C-11′, whereas the other has a double bond at C-12′. Tocomonoenols also have three chiral centres at carbons C-2, C-4′ and C-8′ and all naturally occurring tocomonoenols have the *RRR* configuration. Rhizomes of plants from the genus *Dioscorea* (Dioscoreaceae), known as yams, are a common food in tropical areas and are widely used as a staple medicinal food, for example in the promotion of the health of postmenopausal women. The oestrogenic activity of yams *D. alata* was attributed to α-tocopherol, γ-tocopherol-9 (**5-38**), hydro-Q_9 chromene (**5-39**), coenzyme Q_9 and 1-feruloylglycerol.



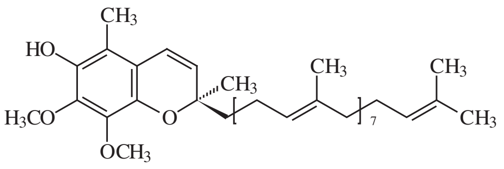
5-36, 3,4-dehydro-α-tocopherol



5-37, α-tocomonoenol isomers



5-38, γ-tocopherol-9



5-39, hydro-Q_9 chromene

5.4.2 Biochemistry

Vitamin E, as well as vitamin K, plastoquinones and ubiquinones, is formed, in principle, from shikimic acid via 4-hydroxyphenylpyruvic and homogentisic acid. The terpenoid side chain is synthesised from isopentenyl diphosphate, or more precisely from geranylgeranyl diphosphate, which is gradually reduced to phytyl diphosphate. The primary product of biosynthesis is δ-tocopherol; other tocopherols are products of its methylation. The biosynthesis of tocotrienols lies in the condensation of homogentisic acid with geranylgeranyl diphosphate. Vitamin E is only synthesised by plants and some cyanobacteria.

The most important lipophilic antioxidant that acts in eucaryotic cells to protect unsaturated lipids against free radical damage is vitamin E, especially α-tocopherol. Along with β-carotene and coenzyme Q, it protects the structure and integrity of biomembranes, such as the cytoplasmic cell membrane (or plasmolema) and intracellular membranes of organelles (nucleus, mitochondria, lysosome and endoplasmic reticulum). It is also employed in the protection of lipoproteins present in plasma. It is transported in the bloodstream by association with the lipid phase of low density lipoprotein (LDL) particles (see Section 3.6.1). Each LDL particle contains six molecules of vitamin E.

Other functions of vitamin E are not yet fully established. Vitamin E probably furthermore directly contributes to the structure of biological membranes and modulation of their properties, because the vitamin specifically interacts with arachidonic acid and has some regulatory function in its metabolism (conversion of arachidonic acid into leucotrienes). Deficiency of vitamin E is manifested in females by placental disorders and in males by disorders of maturation of gametes.

5.4.3 Physiology and nutrition

An adequate intake of vitamin E is believed to prevent oxidation of lipids in biomembranes. Vitamin E is therefore a factor that slows the collection of changes known as the aging process, and is applied in the prevention of cardiovascular diseases and cancer (oncogenesis).

The need for vitamin E is not yet precisely known. It depends greatly on polyunsaturated fatty acid intake through the diet. For people whose average daily intake of these fatty acids is between 14 and 19 g, the recommended daily intake of vitamin E is 15 mg. More (*RRR*)-α-tocopherol (0.5–0.6 mg) is needed for each additional gram of fatty acids taken in. For pregnant women, the recommended daily intake is higher by 2 mg, and for lactating women by 5 mg. The current increasing intake of unsaturated fats

with low oxidation stability highlights the need for a revision of these recommendations. A real need is about 20–30 mg per day. Ideas of the need for higher intake (of around 50 mg, but also up to 100 mg daily) have no basis in scientific knowledge or even in clinical trials.

The international unit of activity (IU) is defined as an activity of 1 mg of synthetic all-*rac*-α-tocopheryl acetate (D,L-α-tocopheryl acetate) that is often used for food fortification and in multivitamin tablets. The biological activity of tocopherols and tocotrienols and their stereoisomers is difficult to determine, and is expressed in a relatively wide range. (*RRR*)-α-Tocopherol is generally considered the most effective substance, and its esters have similar effects. β-Tocopherol has about 50% the activity of α-tocopherol, γ-tocopherol about 10% and δ-tocopherol approximately 3% of the α-tocopherol activity.

Synthetic α-tocopherol exhibits, unlike natural α-tocopherol, lower biological activity, because it consists of the C-2 enantiomers, (2*R*)-α-tocopherol and (2*S*)-α-tocopherol. In short, synthetic α-tocopherol is (2*RS*)-α-tocopherol (D,L-α-tocopherol or 2-*ambo*-α-tocopherol). Synthetic α-tocopherol can also be a racemic mixture of all possible stereoisomers and it then consists of (2*RS*)-, (4′*RS*)- and (8′*RS*)-α-tocopherol (or 2-*ambo*-, 4′-*ambo* and 8′-*ambo*-α-tocopherol), which is called all-*rac*-α-tocopherol.

Commercial vitamin products often contain esters of α-tocopherol (acetate or hydrogen succinate), which are more stable to oxidation compared with free α-tocopherol. However, esterification of the C-6 hydroxyl group (**5-33**) results in the formation of biologically inactive compounds, but these esters are rapidly hydrolysed to the biologically active α-tocopherol under the action of non-specific esterases.

The presence of double bonds in the molecule of the tocotrienols results in a decrease in their biological activity of about one-third compared with tocopherols. Biological activity of any significance is shown only by α-tocotrienol (about 30% of the activity of α-tocopherol) and β-tocotrienol (about 5% of the activity of α-tocopherol).

Vitamin requirements are mainly fulfilled by plant lipids, especially oils, and in some countries by margarine enriched with tocopherols. Other foods of both plant and animal origin are also important additional sources. Although they contain fewer vitamins, they are consumed regularly and in high quantities (e.g. bakery products, meat, eggs and some vegetables).

Vitamin E deficiency is relatively rare, but occasionally occurs in newborns and adolescents. It manifests with similar symptoms as selenium deficiency, because the specific selenoproteins are also involved in transport of tocopherols and protect the tocopherols against oxidation. Manifestation of vitamin E deficiency is mainly through degenerative nerve and muscle (neuromuscular) changes known as myopathy and encephalomalacia. Consumption of excessive amounts of tocopherols occurs only rarely.

5.4.4 Use

For fortification of foods and feeds (such as with vitamins and lipid antioxidants) and for pharmaceutical purposes, synthetic racemic α-tocopherol and its esters or a mixture of natural D-tocopherols obtained as a byproduct of refining (deodorisation) of oils from deodorisation condensates are used. The material produced should be protected from oxidation by addition of a phenolic antioxidant. Tocopherols β, γ and δ can also be converted into α-tocopherol by methylation (tocotrienols by methylation and hydrogenation of the side chain).

5.4.5 Occurrence

Vitamin E is found primarily in foods of plant origin, and to a lesser extent (with a few exceptions) in foods of animal origin. Other sources do not have any practical significance (Table 5.5).

All eight biologically active tocopherols and tocotrienols occur in foods. Unsubstituted tocol does not occur in nature. Unsubstituted tocotrienol (also called desmethyl tocotrienol) was recently isolated from heated rice bran together with so-called didesmethyl tocotrienol, which lacks the C-2 methyl group. The main compounds are α-, β-, γ- and δ-tocopherol and α-, β- and γ-tocotrienol. Tocotrienols are also found in the form of esters.

5.4.5.1 Foods of animal origin

In animal tissues, the composition of vitamers E is affected mainly by the feed composition. Vitamin content also varies according to the season. The main component (more than 90% of vitamin E) is always α-tocopherol. Animal fats contain much less vitamin E than vegetable oils. The total content of vitamin E in butter is up to 50 mg/kg (γ-tocopherol content is up to 10% of the total vitamin E content), in pork fat 6–30 mg/kg (about 5% as γ-tocopherol, 5% as α-tocotrienol), in beef tallow up to 20 mg/kg and in chicken fat up to 25 mg/kg. Unlike other lipophilic vitamins, vitamin E does not occur in large quantities in fish oils. The content in cod liver oil is only 0.25 mg/kg.

5.4.5.2 Foods of plant origin

In cereals, vitamin E is located mainly in the germ and bran, so white flours have lower vitamin content than whole grain flours.

Table 5.5 Vitamin E content of selected food commodities.

Food	Edible portion (mg/kg or mg/l)	Food	Edible portion (mg/kg or mg/l)
Meet	2.5–7.7	Soybeans	2.7–13
Liver	4–14	Apples	1.8–7.4
Milk	0.2–1.2	Oranges	2.4–2.7
Butter	10–50	Cabbage	0.2–11
Cheeses	3.0–3.5	Spinach	16–25
Eggs	5–30	Tomatoes	3.6–4.9
Fish	4–80	Carrots	2.5–4.5
Flour (wheat)	15–50	Potatoes	0.6–0.9
Rice	0.4–4.5	Walnuts	35

Table 5.6 Tocopherols and tocotrienols content of cereals, pseudocereals and plant oils (in mg/kg).

Vitamin	Wheat	Rye	Barley	Oat	Maize	Rice	Millet	Wheat bran oil	Rapeseed oil	Olive oil
α-Tocopherol	9.7-14.0	8.0-16.0	3.7-11.6	4.3-8.9	0.2-22.1	0.6-7.5	0.8	1328	300	93-260
β-Tocopherol	3.9-7.0	2.2-4.0	0.2-0.7	0.6-0.9	0.4	0.7	0.5	505		1.2-3.4
γ-Tocopherol	-	6.0	0.2-12.9	0.9	17.5-56.0	0.8-4.0	23.3	112	481	2.6-9.8
δ-Tocopherol	-	-	0.1-0.9	-	0.3-3.8	0.2-0.5	6.1	51	29	0.1-0.5
α-Tocotrienol	2.4-5.0	12.5-15.0	13.0-36.0	11.0-25.2	0.3-2.6	1.0-4.1	<0.2	40	<10	0.3-1.0
β-Tocotrienol	19.0-33.0	7.0-11.8	2.7-14.3	0.9-3.3	-	<0.2	-	140	-	-
γ-Tocotrienol	-	<0.2	3.6-8.4	0.2	3.3-9.9	2.0-9.5	0.7	-	-	0.4-0.9
δ-Tocotrienol	-	-	0.7-3.9	0.2	-	0.2-0.7	-	-	-	-
Total	35-59	32-44	31-80	19-38	22-94	4-27	31.6	2180	820	100-270

The total vitamin E content is about 15–50 mg/kg. Details of the main forms of vitamin E in cereals and pseudocereals are shown in Table 5.6. The main form of vitamin E in the green parts of plants is α-tocopherol, located in the plastids; γ-tocopherol is the major form of vitamin E in cells that do not have chloroplasts where photosynthesis does not occur (e.g. in seeds).

Vegetable oils have a somewhat lower content of vitamin E than oils from germs. Virgin (crude) oils, contain higher amounts of vitamin E than refined oils. The vitamin E content in crude rapeseed oil, for example, is 360–1000 mg/kg; the refined oil vitamin content is 140–850 mg/kg. Crude and refined sunflower oils have vitamin E contents of 270–1240 and 270–900 mg/kg, respectively. Among the common vegetable oils, soybean oil contains the highest amount of vitamin E (530–2000 mg/kg); 11% of the vitamin is in the form of α-tocopherol, over 60% in the form of γ-tocopherol and more than 20% as δ-tocopherol, with tocotrienols present in negligible quantities. The vitamin E content of olive oil is about 160 mg/kg.

Germ oils contain large amounts of vitamin E. For example, wheat germ oil contains 1650–3000 mg/kg of vitamin E, which consists of about 61% of α-tocopherol, 23% of β-tocopherol, 5% of γ-tocopherol, 2% of δ-tocopherol and approximately 8% of tocotrienols, of which β-tocotrienol is the dominating form (Table 5.6).

The content of vitamin E in fruits and vegetables usually does not exceed 10 mg/kg. The main vitamin form is α-tocopherol. Apples contain, on average, 1.8 mg/kg of α-tocopherol, but 6.1 mg/kg are found in the skin. The vitamin content in cabbage is about 0.9 mg/kg, of which only 0.1 mg/kg represents α-tocopherol, the rest is δ-tocopherol. Carrots contain about 2.5 mg/kg of α-tocopherol, 0.2 mg/kg of β- and γ-tocopherol and 0.1 mg/kg of δ-tocopherol. Lettuces contain about 3.2 mg/kg of α-tocopherol, 1.5 mg/kg of β-tocopherol and 2 mg/kg of γ-tocopherol. Potatoes contain very little vitamin E (about 0.1 mg/kg of α-tocopherol in the flesh and skin). Even tubers from transgenic plants (containing a gene or genes transferred from another species) accumulate approximately 10–100-fold less α-tocopherol than leaves or seeds. Relatively high amounts of vitamin E are found, of course, in potatoes fried in oil; for example, potato chips with reduced fat content (20.8%) contain about 55 mg/kg of α-tocopherol.

5.4.6 Reactions

Tocopherols and tocotrienols are monoethers of the respective hydroquinones and are therefore readily oxidised, for example by ferric ions, lipid hydroperoxides and other oxidants. This creates the corresponding quinones (tocopheryl quinones or tocoquinones). Tocopheryl quinones can be reduced to tocopheryl hydroquinones (tocohydroquinones). The most important reactions are those with oxidised lipids (Figures 5.7 and 5.8).

Biological activity of vitamin E is related to antioxidant effects; the most effective antioxidant (*in vivo*) is α-tocopherol. In food lipids, the situation is more complicated, because the antioxidant activity of tocopherols and tocotrienols depends on many factors. One important aspect is the amount and composition of unsaturated fatty acids. Under typical storage conditions, tocopherols are more effective antioxidants, for example, in animal fats (the main fatty acid is oleic acid) compared with vegetable oils that contain higher amounts of linoleic acid (e.g. soybean and sunflower oils).

The antioxidant activity of vitamin E in emulsions depends on the structure of the emulsions and the presence of other antioxidants, such as 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) and ascorbyl palmitate. Temperature plays an important part, as does, particularly, the presence of oxygen and the stability of the radicals of tocopherols produced as intermediates in reactions with oxidised lipids. At 80 °C in the presence of air, δ-tocopherol, for example, is the only vitamin form which partially withstands heating for 6 h, when used as an antioxidant to protect linoleic acid against autoxidation. In an atmosphere containing only 10% oxygen (half of the amount of oxygen in air), β- and γ-tocopherols are also present, but α-tocopherol and all tocotrienols are absent. At 60 °C in the absence of oxygen, all tocopherols and tocotrienols are present.

For all these reasons, therefore, the following relative order of antioxidant efficiency of tocopherols in food is generally given, which is the opposite to the order of their biological activities

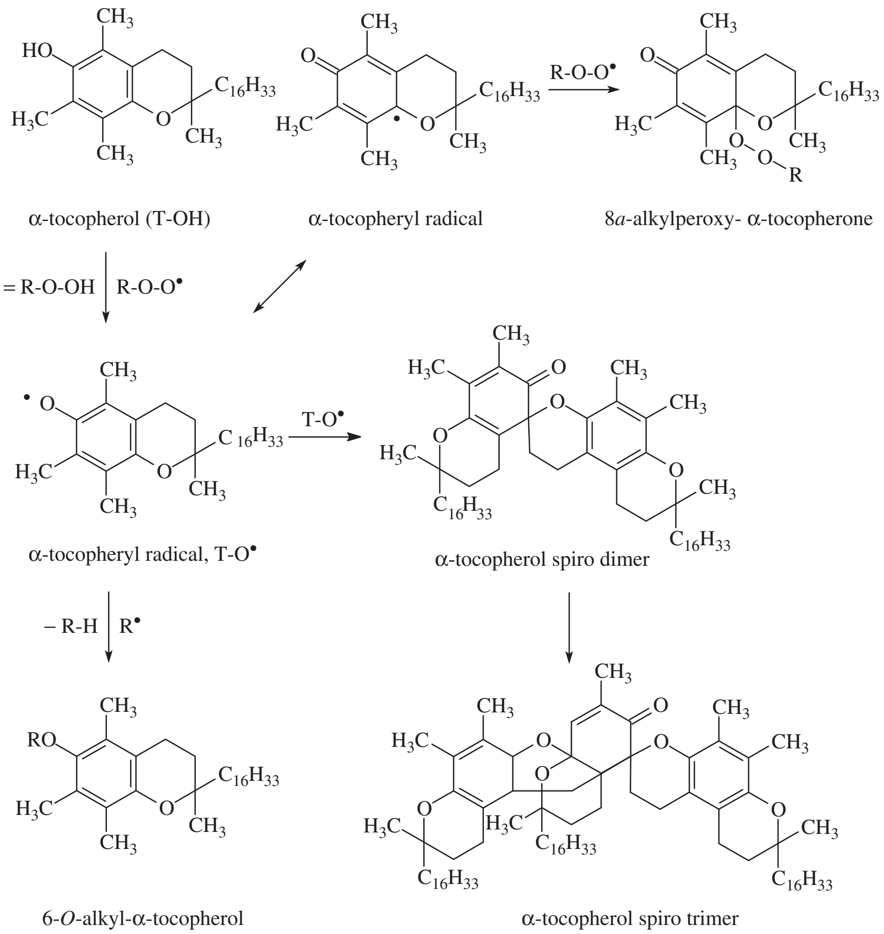


Figure 5.7 Main reaction products of α-tocopherol with oxidised lipids.

(tocotrienols are less effective than the corresponding tocopherols):

$$\delta\text{-T} > \gamma\text{-T} > \beta\text{-T} > \alpha\text{-T or } \delta\text{-T} > \gamma\text{-T} = \beta\text{-T} > \alpha\text{-T}$$

The mechanism of the antioxidant effect of vitamin E is similar to the effect of other lipophilic antioxidants. Tocopherols react with a number of free radicals including active oxygen species. One tocopherol molecule can react with two hydroperoxyl radicals. Autoxidation of lipids is inhibited by reaction of tocopherols (abbreviated as T–OH) with hydroperoxyl lipid radicals (R–O–O$^\bullet$) with the formation of hydroperoxides (R–O–OH) and radicals of tocopherols (tocopheroxyl radicals, T–O$^\bullet$). This reaction interrupts the radical chain autoxidation reaction of lipids during the propagation phase:

$$\text{R–O–O}^\bullet + \text{T–OH} \rightarrow \text{R–O–OH} + \text{T–O}^\bullet$$

The resulting tocopheroxyl radical is not sufficiently reactive and therefore cannot split other lipid (fatty acid) molecules. In the termination phase of the chain autoxidation, the tocopheroxyl radical stabilises by irreversible reactions with other radicals, mostly with the second hydroperoxyl radical:

$$\text{T–O}^\bullet + \text{R–O–O}^\bullet \rightarrow \text{stable products}$$

Both reactions occur in lipids containing low concentrations of tocopherols and also in the presence of sufficient amounts of oxygen. Alternatively, other reactions take place. In the presence of large quantities of tocopherols, some tocopheroxyl radicals react with each other to form a dimer or trimer:

$$\text{T–O}^\bullet + \text{T–O}^\bullet \rightarrow \text{tocopherol dimer}$$

With an adequate supply of oxygen, the hydrocarbon radicals (R$^\bullet$), formed in the initial phase of the autoxidation chain reaction, preferentially yield hydroperoxyl radicals (ROO$^\bullet$). When there is a limited supply of oxygen (with a low partial pressure of oxygen) and in the absence of antioxidants, hydrocarbon radicals react with each other and form lipid dimers (R–R). In the presence of tocopherols, there is a competitive reaction of the hydrocarbon radicals with tocopheroxyl radicals that are stabilised by the formation of hydrocarbons (R–H) and other stable products:

$$\text{R}^\bullet + \text{R}^\bullet \rightarrow \text{R–R}$$
$$\text{R}^\bullet + \text{T–OH} \rightarrow \text{R–H} + \text{T–O}^\bullet$$
$$\text{R}^\bullet + \text{T–O}^\bullet \rightarrow \text{stable products}$$

Figure 5.8 shows, as an example, the most important products of α-tocopherol in reactions with oxidised lipids. The main products,

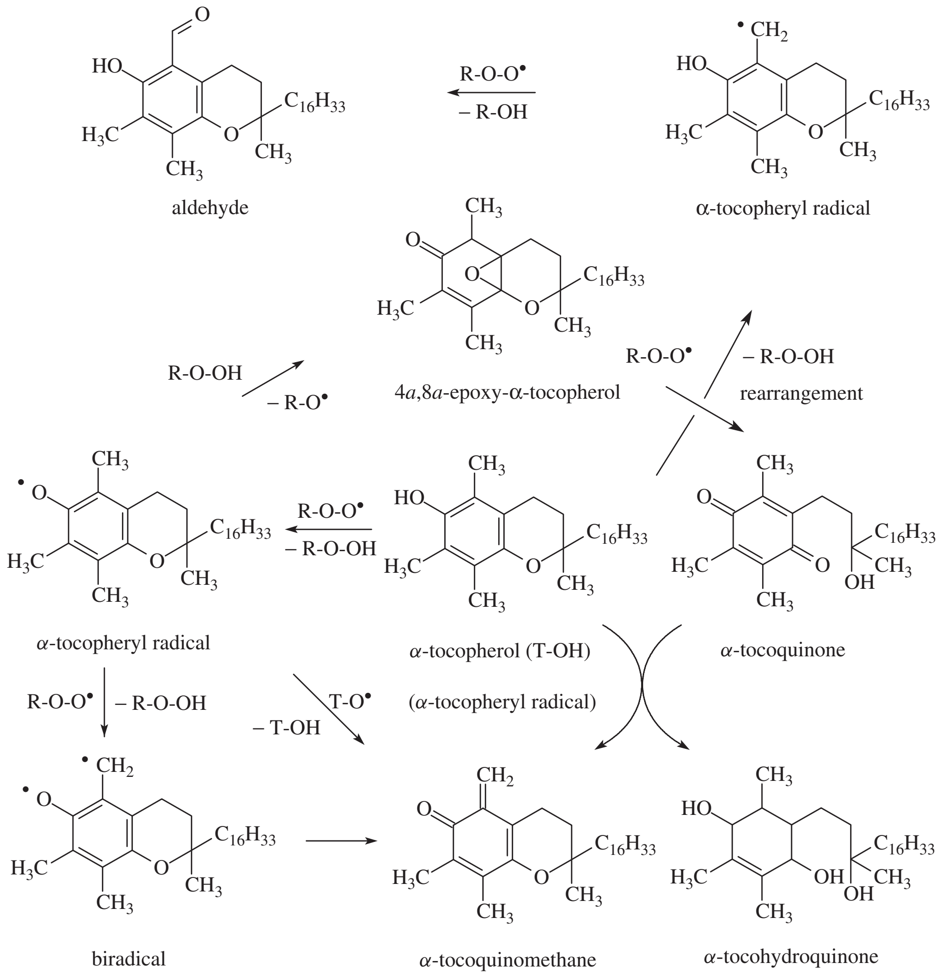
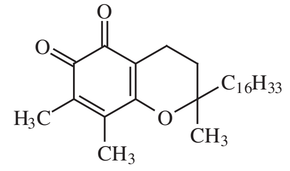


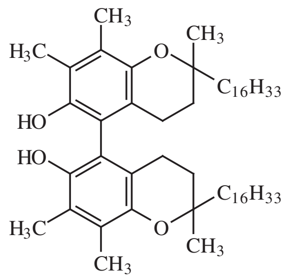
Figure 5.8 Minor reaction products of α-tocopherol with oxidised lipids.

formed by reaction of tocopheroxyl radicals with hydroperoxyl radicals, are 8a-alkylperoxy-α-tocopherons.[5] To a lesser extent the recombination of tocopheroxyl radicals with hydrocarbon radicals (and alkoxyl radicals, RO•) also occurs. The reaction products are the corresponding 6-*O*-alkyl-α-tocopherols. Dimers (which show reducing effects as well as tocopherols), trimers and other products, such as epoxides, quinones, hydroquinones, qinomethanes[6] and other compounds are likewise formed as minor products.

Similarly to α-tocopherol, other tocopherols and tocotrienols also react with oxidised lipids Some reaction products of γ-tocopherol, such as tocopherol red (tocored, **5-40**, arising during bleaching of vegetable oils), C–C dimer (**5-41**), C–O–C dimer (**5-42**) and trimer (**5-43**), do not arise from α-tocopherol.



5-40, tocopherol red



5-41, γ-tocopherol C–C-dimer

Tocopherols can also react with singlet oxygen as quenchers (analogously to β-carotene) with the formation of various oxidation products. The primary reaction is probably 1,4-cycloaddition of oxygen to form endoperoxides (Figure 5.9). If the phenolic group of the tocopherols is located at the lipid–water interface, tocopheroxyl radicals present in the lipid phase can be reduced

[5]For example, oxidised linoleic acid esters (hydroperoxyl radicals of this acid) yield a mixture of (9*R*,8a*R*)-, (9*R*,8a*S*)-, (9*S*,8a*R*)-, and (9*S*, 8a*S*)-9-(8a-peroxy-α-tocopheron)-(10*E*,12*Z*)-octadeca-10,12-dienoate together with (13*R*,8a*R*)-, (13*R*,8a*S*)-, (13*S*,8a*R*)-, and (13*S*,8a*S*)-13-(8a-peroxy-α-tocopheron)-(9*Z*, 11*E*)-octadeca-9,11-dienoate.

[6]Quinomethanes (formerly known under incorrect names quinone methides or quinomethides) are methylidenecyclohexadienones and dimethylidenecyclohexadienes, formally derived from quinones by replacement of one or both of the quinone oxygen atoms by a methylidene group.

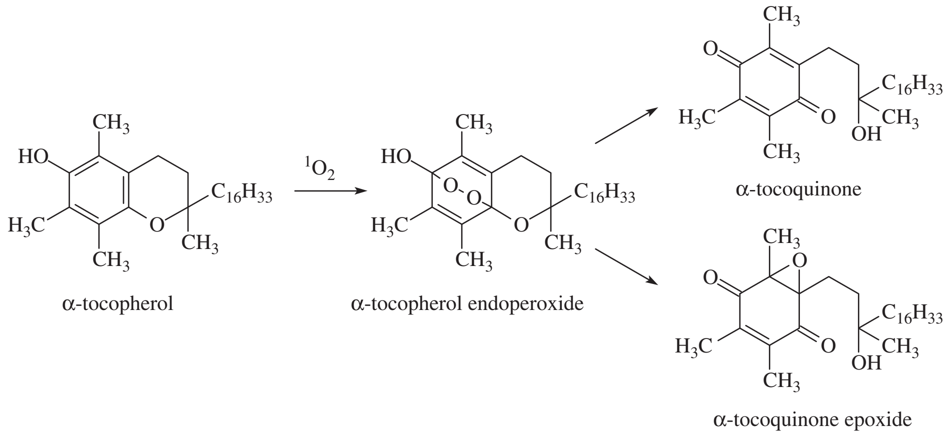
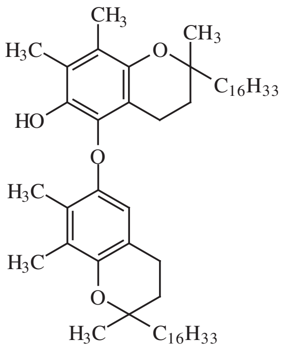
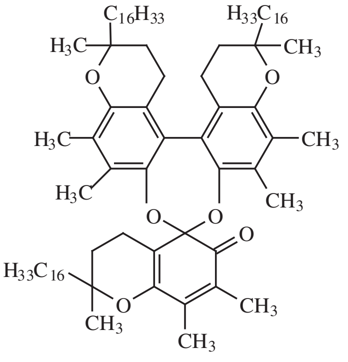


Figure 5.9 Oxidation of α-tocopherol by singlet oxygen.



5-42, γ-tocopherol C–O–C dimer



5-43, γ-tocopherol trimer

back to tocopherols by water-soluble ascorbic acid, which thus also protects vitamin E against oxidation *in vivo*.

5.4.7 Changes in foods

5.4.7.1 Fats and oils

The refining of edible oils reduces the vitamin content to 10–50% of the original amount. The major losses occur during the deacidification step (removal of free fatty acids), if this step is performed by chemical methods (due to oxidation of vitamin E in the alkaline medium). High losses of vitamin E also appear during oil bleaching (oxidation on the surface of bleaching earths catalysed mainly by ferric ions). Losses during the deodorisation step are mainly caused by volatilisation of vitamin E with water vapour under reduced pressure. Hydrogenation of fats using nickel catalysts (Raney nickel) leads to losses of 30–50%.

5.4.7.2 Other foods

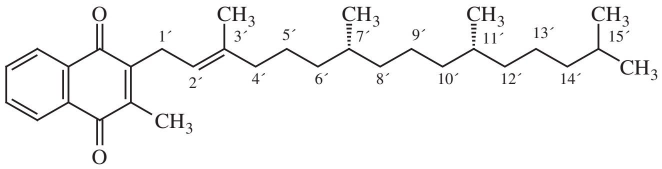
In the absence of oxygen and oxidised lipids (their hydroperoxides), vitamin E is relatively stable during normal culinary and industrial processing of foods. During processing and storage of meat, meat products, milk, dairy products and cereals, the losses usually do not exceed 10% of the original amount. For example, the loss of vitamin E during pasteurisation of milk is about 5%. Stored grains lose about 10% of their vitamin content per month. The largest losses occur during frying and baking. In fats that are used repeatedly for frying food, tocopherols are virtually absent, because they are volatile along with the water vapour and decompose at higher temperatures. An analogous situation is in fried, frozen and stored products, such as pre-fried potato chips (French fries). In general, the vitamin E content gradually decreases even under refrigerated storage of foods containing higher amounts of polyunsaturated fatty acids. Drying of fruits or vegetables results in a loss of 50–70% of vitamin E.

5.5 Vitamin K

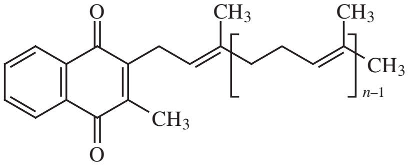
5.5.1 Structure and terminology

All naturally occurring compounds that show vitamin K activity (the coagulation vitamin) are derivatives of menadione (2-methyl-1,4-naphthoquinone) with an isoprenoid unsaturated side chain at the C-3 position of the aromatic ring. Today, essentially two types of active substances are recognised. The other compounds with vitamin K activity are synthetic derivatives.

Vitamin K_1, also known as vitamin $K_{1(20)}$, phylloquinone or phytylmenaquinone, contains a hexahydrotetraprenyl chain (2-methyl-3-phytyl-1,4-naphthoquinone, **5-44**). Phylloquinone occurs in foods of plant origin. The side chain derived from phytol has 20 carbon atoms (four isoprenoid units, of which three are reduced). The isomer with a *cis*-configuration in the side chain is not biologically active. The systematic name of vitamin K_1 is (2′*E*,7′*R*,11′*R*)-2-methyl-3-(3′,7′,11′,15′-tetramethylhexadecyl)naphtho-1,4-quinone. The second compound with the activity of vitamin K is vitamin K_2 (**5-45**), also known as vitamin $K_{2(n)}$, menaquinone, menaquinone-*n*, MK-*n* or 2-methyl-3-multiprenyl-1,4-naphthoquinone ($n = 0$–13). The most common menaquinones contain 4–10 isoprene units, such as MK-7, additionally called pharnoquinone. Vitamin K_2 is produced by many bacteria and actinomycetes. A multiprenyl side chain



5-44, phylloquinone



5-45, menaquinone (n = 0–13)

with an all-*trans* configuration is the most common substituent. The bacteria usually produce menaquinone with different chain lengths. The main menaquinone of *Escherichia coli* is, for example, MK-8, *Staphylococcus aureus* produces menaquinones MK-0 to MK-9. One or more isoprenoid groups in the side chain can be hydrogenated. Some bacteria also produce related 2-demethylmenaquinones. Yeasts do not produce vitamin K_2, but only related coenzymes Q.

Vitamin K_3 (menadione, MK-0, 2-methyl-1,4-naphthoquinone) is a synthetic substance. The product of menadione reduction, known as menadiol (2-methylnaphthalene-1,4-diol), and derived compounds, such as the fat-soluble menadiol diacetate or menadiol dibutyrate and the water-soluble sodium salt of menadiol diphosphate, are referred to as vitamin K_4. Activity of vitamin K was also detected in the synthetic monoamino analogues and diamino analogues of menadiol, for example, in 1-amino-4-hydroxy-3-methylnaphthalene (vitamin K_5), 1,4-diamino-2-methylnaphthalene (vitamin K_6) and 1-amino-4-hydroxy-2-methylnaphthalene (vitamin K_7).

5.5.2 Biochemistry

Phylloquinone and menaquinone are derived from chorismic acid, which results from 3-phosphoenolpyruvic acid (a product of glycolysis) and D-erythrose 4-phosphate (a product of the pentose and Calvin cycles) as a starting compound for the biosynthesis of phenylalanine, tyrosine and tryptophan. It is transformed into isochorismic acid; other carbon atoms are derived from 2-oxoglutaric acid. The side chain is provided by phytyl diphosphate or by polyprenyl diphosphates, which are formed from geranylgeranyl diphosphate. The final reaction is a methylation at C-2.

Vitamin K in birds and mammals occurs in the reduced form (as a hydroquinone) and acts as an essential factor for the carboxylation of certain proteins (transformation of bound glutamic acid into γ-carboxyglutamic acid). Carboxyglutamic acid residues impart important properties to the relevant proteins, such as the ability to bind calcium ions and phospholipids, which are necessary for their activation and for them to function in blood clotting. The best known reaction is the conversion of inactive prothrombin into the active proteolytic enzyme thrombin.

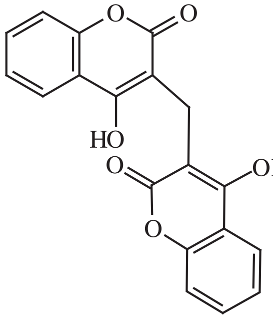
The main transport form in plasma is vitamin $K_{1(20)}$, which is bound to very low density lipoproteins (VLDL). In the liver, about 90% of vitamin K is represented as menaquinones MK-7 to MK-12.

5.5.3 Physiology and nutrition

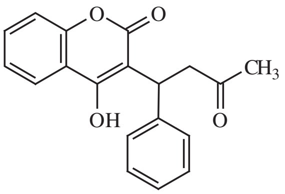
The daily requirement of vitamin K is estimated to be between 0.01 mg (for babies) and 0.14 mg (for adults). The daily intake by food is estimated at 0.3–0.5 mg, but only about 30–70% of dietary vitamin is absorbed in the intestines. According to some data, about 40–50% of the daily requirement of the vitamin is provided by foods, and under normal circumstances intestinal microflora produces the rest.

A deficiency in the production of vitamin K may occur during intestinal dysbiosis (an impaired balance of the intestinal microflora), in inflammatory intestinal diseases (such as Crohn's disease), in intestinal absorption disorders and with an inadequate production of bile. Deficiency can be manifested by blood clotting disorders (this is rare in humans). Chickens are particularly sensitive to the lack of vitamin K, mainly due to the use of antibiotics that inhibit the activity of intestinal microflora. Vitamin K is therefore added to feed mixtures to prevent bleeding into the muscles and skin.

Vitamin K antagonists (anticoagulants) are typically coumarins, and particularly dicoumarol (also known as melitoxin, **5-46**), which is produced in larger concentrations in the mouldy clover from 2-coumaryl-CoA under the action of fungi enzymes. Warfarin, that is 3-(3-oxo-1-phenylbutyl)-4-hydroxycoumarin (**5-47**), which also shows a significant anticoagulant effect, is used as a rodenticide (a chemical intended to kill rodents), but also in human medicine to prevent increased blood clotting, which can lead to embolisms. The mechanism of action of anticoagulants is that of competitive inhibition of the conversion of 2,3-epoxyvitamin K into vitamin K in the liver.



5-46, dicoumarol

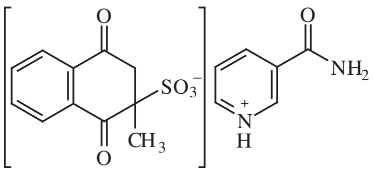


5-47, warfarin

5.5.4 Use

Vitamin K_3 and its water soluble forms, such as salts or complexes of its addition product with sodium hydrogen sulfite (trihydrate of sodium salt), and its complex with nicotinamide (**5-48**) or with

other amino compounds, are used as additives in the fattening of chickens.



5-48, menadionbisulfite/nicotinamide complex

5.5.5 Occurrence

5.5.5.1 Foods of animal origin

Meat and meat products have a moderately high vitamin K content. Liver is high in vitamin K (Table 5.7). In pigs' liver, for instance, more than ten active substances have been identified, of which vitamin $K_{1(20)}$ (0.012 mg/kg), MK-4 (0.011 mg/kg), MK-7 (0.016 mg/kg), MK-8 (0.025 mg/kg), MK-9 (0.006 mg/kg) and MK-10 (0.008 mg/kg) occur in significant quantities. Menaquinones MK-10 to MK-13, originally synthesised by bacteria in the rumen and subsequently absorbed, have been found in beef liver.

5.5.5.2 Foods of plant origin

Vitamin $K_{1(20)}$ is the only form of vitamin K that is found in foods of plant origin. It is a normal constituent of specialised cells for photosynthesis (chloroplasts), whereas in foods of animal origin, more active forms of vitamin K are present. Rich sources of vitamin K are mainly green leafy vegetables. For example, green cabbage leaves from the edge of the cone contain 3–6 times higher amounts of vitamin K than the yellow leaves inside the cones. Vegetable oils are also good sources of vitamin K. Fruits, potatoes and cereals are low in vitamin K.

Table 5.7 Vitamin K content of some foods.

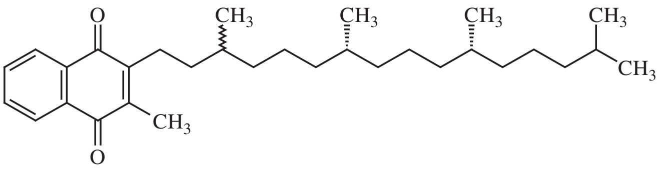
Food	Edible portion (mg/kg or mg/l)	Food	Edible portion (mg/kg or mg/l)
Pork liver	0.08	Cabbage	1.1–2.5
Beef liver	0.14	Broccoli	1.5–1.8
Pork meat	0.03	Spinach	2.0–14.4
Beef meat	0.04	Beans	0.1–0.5
Chicken meat	0.60	Peas	0.4
Fish (rainbow trout)	0.04	Tomatoes	0.02–0.06
Fish (Baltic herring)	0.01	Carrot	0.01–0.05
Eggs	0.02	Potatoes	0.01–0.02
Milk (fresh)	0.01–0.03	Strawberry	0.01
Yogurt (plain)	0.01	Soybean oil	1.39–2.90
Cheese (Edam type)	0.49	Rapeseed oil	1.14–1.88
Cheese (Emmental type)	0.08–0.09	Olive oil	0.3–0.8
Bread	0.004	Sunflower oil	0.09

5.5.5.3 Other sources

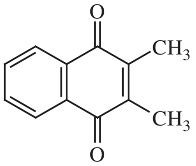
Vitamin K_2 is produced mainly by the bacteria *Escherichia coli* and bacteria of the genus *Bacillus*. In bacteria and actinomycetes it occurs at levels of about 600–1700 mg/kg dry matter. The synthetic menadione and its derivatives are transformed by bacteria into MK-4 with a tetraprenyl side chain. This form of vitamin K also occurs in the tissue of animals whose feed was fortified with this vitamin.

5.5.6 Reactions

Adverse reactions and loss of vitamin K activity occurs under exposure to light (photodegradation to a number of products), in reactions with reducing agents and in alkaline media. The hydroquinone forms of the vitamin are oxidised to quinones by the air oxygen. Oxidation of phylloquinone (e.g. by hydrogen peroxide in an alkaline medium) yields 2,3-epoxide. During hydrogenation of vegetable oils, the side chain of phylloquinone is also partly hydrogenated. The hydrogenated fat contains the original phylloquinone, partly 2′,3′-dihydrophylloquinone (dihydrovitamin K_1, **5-49**) and decomposition products of phylloquinone, such as 2,3-dimethyl-1,4-naphthoquinone (**5-50**). The original vitamin content is decreased by 50% or more.



5-49, dihydrovitamin K_1



5-50, 2,3-dimethyl-1,4-naphthoquinone

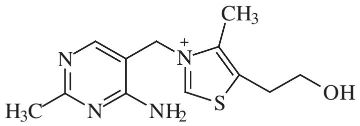
5.5.7 Changes in foods

During storage and thermal processing of foods, vitamins K are relatively stable. Significant losses, however, occur when food is exposed to daylight. The vitamin content in vegetable oils decreases during frying (for 30 min at 190 °C), for example, to 85–90% of the original amount. If oil is exposed to daylight at ambient temperature, the vitamin content decreases to 50% in one day.

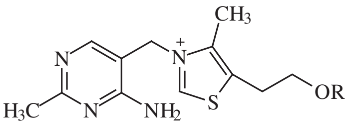
5.6 Thiamine

5.6.1 Structure and terminology

Thiamine (vitamin B_1, also formerly known as aneurine) contains a pyrimidine ring (4-amino-2-methylpyrimidine) attached by the methylene group at C-5 to the nitrogen of 5-(2-hydroxyethyl)-4-methylthiazole. Thiamine (**5-51**) occurs primarily as a free compound and in the form of phosphate esters (**5-52**), the monophosphate, diphosphate (pyrophosphate called cocarboxylase) and triphosphate.



5-51, thiamine (free base)



5-52, thiamine phosphates

thiamine phosphate, R = PO_3H_2
thiamine diphosphate, R = $P_2O_6H_3$
thiamine triphosphate, R = $P_3O_9H_4$

5.6.2 Biochemistry

Thiamine is synthesised *de novo* by all higher plants and many microorganisms. However, some microorganisms synthesise only the pyrimidine moiety (using 5-aminoimidazole ribonucleotide, known as AIR; an intermediate in the metabolism of purines) or only the thiazole part (from histidine and pyridoxol), and the remaining part of the thiamine molecule is obtained from the environment. Animals do not synthesise thiamine. Free thiamine obtained from food (esters of thiamine after prior hydrolysis) is esterified to thiamine diphosphate, the active form of thiamine, in cells of various organs by pyrophosphokinase. Thiamine diphosphate is a cofactor of many important enzymes (such as decarboxylases, dehydrogenases, transketolases and carboligases) associated primarily with the metabolism of carbohydrates and branched chain aliphatic amino acids. Thiamine diphosphate bound to proteins is then esterified to thiamine triphosphate.

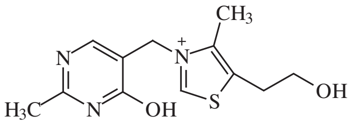
5.6.3 Physiology and nutrition

Thiamine is a cofactor of enzymes involved in energy metabolism, therefore the required amount of the vitamin is mainly related to the amount of utilisable carbohydrates (D-glucose) received in the food. For every 4200 kJ (1000 kcal) of energy derived from carbohydrates, an intake of 0.4–0.6 mg of thiamine is recommended. For adults with a daily energy intake of 12 600 kJ (3000 kcal), the recommended intake of thiamine is 1.2 mg (for infants typically 0.3 mg, 0.7–1.2 mg for children, 1.2–1.5 mg for males, 1.0–1.1 mg for women, 1.4 mg for pregnant women and 1.5 mg for lactating women).

Thiamine is, like some other vitamins, produced by intestinal microflora. The level of vitamin delivered in this way is too low, so in practice the required amount is only obtained through food. The most important sources of thiamine are whole grain cereal products that supply about 40% of the vitamin requirements (bread covers about 20%). Other important sources are meat and meat products (18–27%), milk and dairy products (8–14%), potatoes (10%), legumes (for reasons of low consumption, about 5%), vegetables (up to 12%), fruits (about 4%) and eggs (about 2%). Other foods supply the requirements for this vitamin to a lesser extent.

Thiamine deficiency is manifested by non-specific symptoms such as muscle fatigue, anorexia, weight loss and irritability. The reason is the partial oxidation of glucose to pyruvic acid. Vitamin deficiency may appear as a result of its increased demand (e.g. during pregnancy, lactation and sport activities), through significantly inadequate or faulty nutrition, artificial (parenteral) nutrition over a long period of time, reduction in the diet, haemodialysis, treatment with antibiotics, which suppress the intestinal flora that synthesise B group vitamins, and chronic alcoholism. A deficient intake brings about alcoholic cardiomyopathy, the Wernicke–Korsakoff syndrome (encephalopathy and psychosis) or beriberi (a neurological and cardiovascular disease).

Thiamine has a high specificity, which means that even small changes of the molecule lead to a reduction of the vitamin effect, inefficiency and in some cases even to an antivitamin effect. A well-known thiamine antagonist is oxythiamine (**5-53**), which contains as a substituent of the pyrimidine ring hydroxyl group instead of an amino group. Oxythiamine is formed from thiamine in strongly acidic media and therefore occurs, for example, in acid protein hydrolysates used as soup seasonings. Oxythiamine easily forms diphosphate, which is a competitive inhibitor of thiamine in enzymatic reactions.



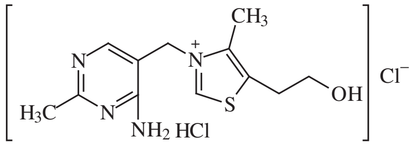
5-53, oxythiamine (free base)

Thiaminases are enzymes that are significant antivitamins, as are some low molecular weight substances from plant foods, such as phenolic compounds in blueberries, red currants, red cabbage and Brussel sprouts. Higher activity of thiaminase I is seen in raw meat and some fresh raw sea fish, molluscs, the raw offal of farm animals and some plants. Thiaminase II is an enzyme present in many bacteria.

5.6.4 Use

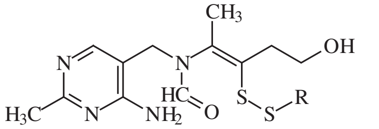
In some countries, white wheat flour, breakfast cereals and rice are fortified with thiamine. For further processing in the pharmaceuticals and food industries, the most commonly used

compound is synthetic thiamine chloride hydrochloride, also called thiamine hydrochloride (**5-54**).



5-54, thiamine chloride hydrochloride

Vitamin activity is also seen in some lipophilic thiamine derivatives with an opened thiazole ring (disulfides derived from the thiol form of thiamine). Oxidative cleavage of the thiazole ring in alkaline solution yields thiamine thiol, which can react with other thiols, forming thiamine alkyl disulfides known as allithiamines (**5-55**). A number of allithiamines occur in plants, especially in members of the genus *Allium* (family: Amaryllidaceae, subfamily: Allioideae). Thiamine allyl disulfide is a lipid-soluble form of thiamine that occurs naturally in garlic. In higher quantities it forms via the heating of thiamine with garlic extract at a temperature of about 60 °C. Garlic extract contains allicin, a diallyl disulfide that is produced by degradation of the amino acid (+)-*S*-allyl-L-cysteine sulfoxide (known as alliin). Allithiamines are biologically active compounds as, on reductive cleavage of the disulfide bridge, they spontaneously dehydrate to yield thiamine. Some synthetic allithiamines (such as thiamine propyl disulfide) have been used for the prevention and treatment of thiamine deficiency.



5-55, allithiamines

thiamine allyl disulfide, R = CH_2–CH=CH_2
thiamine propyl disulfide, R = CH_2–CH_2–CH_3

5.6.5 Occurrence

Free thiamine and its phosphoric acid esters occur in all foods, but only in some are they present in significant amounts (Table 5.8). In general, higher concentrations of thiamine (1–10 mg/kg) are found in foods that are rich in carbohydrates, which undergo intensive metabolism of sugars (cereals and legumes, as well as in pork meat and liver).

5.6.5.1 Foods of animal origin

Animal tissues contain approximately 80–90% of the vitamin as thiamine diphosphate bound to proteins. Pork and chicken meat also contain higher concentrations of thiamine triphosphate (70–80% of the total vitamin content). A particularly rich source of thiamine is pork meat (which contains about ten times more thiamine than other types of meat), ham and other pork meat products, as well as other types of meat, milk, dairy products and eggs.

Thiamine is present in milk mainly as a free compound and the diphosphate is partly bound to proteins. Cows' milk contains about 50–75% of free thiamine and 18–45% of phosphorylated thiamine, which is more labile than free thiamine. About 5–17% of thiamine phosphates are bound to proteins. These forms contribute to about 90% of the activity of free thiamine.

5.6.5.2 Foods of plant origin

In cereals, legumes and generally all plant seeds, the most common form of thiamine is free thiamine. In cereals, which are the most important source of thiamine due to their large consumption, thiamine is found mostly in the outer layers (aleurone layer) of the grain and in the germ, which are removed during the milling process, and thus a large proportion of the thiamine is in the bran. White flours, therefore, contain about ten times less thiamine than whole grain flours. Whole grain cereal products that are rich in thiamine, however, also contain relatively high concentrations of fibre and phytates (see Section 6.2.2.3), which inhibit intestinal absorption of thiamine. Brown rice (or hulled rice), which is unmilled or partly milled, contains a higher amount of thiamine than white rice, which is milled (with the bran and germ removed). Parboiled rice contains a higher amount of thiamine than white rice, as it is absorbed during the process of parboiling. In this process, the harvested rice (rice with husk) is hydrated and then steamed, before drying. Once dried completely, the husk is removed. As a result, the majority of thiamine is absorbed in the inner parts of the grain. Eating brown and parboiled rice is therefore important in preventing of avitaminosis. Another important source of thiamine is potatoes and legumes.

5.6.5.3 Other sources

Brewer's yeast is a rich source of thiamine, because the cells absorb thiamine present in the malt (containing about 160 mg/kg thiamine). Thus this means that in beer, the thiamine concentration is very low (0.01–0.06 mg/l).

5.6.6 Reactions

Thiamine is among the least stable of the vitamins. The reaction catalysed by thiaminase I, which is, for example, present in raw fish, lies in the cleavage of the thiamine molecule by an exchange reaction with nitrogen bases (such as nicotinic acid) or thiols (such as cysteine). Thiaminase II (found in some microorganisms) catalyses thiamine hydrolysis with the formation of the same products that form by non-enzymatic hydrolysis (Figure 5.10).

Thiamine is relatively stable in acidic solutions (pH < 5). Thiamine diphosphate is unstable in weakly acidic and neutral solutions, and its hydrolysis yields thiamine monophosphate and thiamine. In neutral and alkaline solutions, thiamine exists as the free base, which is very unstable. It is hydrolysed to 4-amino-5-hydroxymethyl-2-methylpyrimidine and 5-(2-hydroxyethyl)-

Table 5.8 B group vitamins content of common foods.

Food	Edible portion (mg/kg or mg/l)						
	Thiamine	Riboflavin	Niacin	Pyridoxine	Pantothenic acid	Biotin	Folacin
Pork meat	3.9-11	0.9-3.5	18-130	0.8-6.8	3.0-30	0.05	0.01-0.04
Beef meat	0.4-1.0	0.4-3.5	38-102	0.8-5.0	3.0-20	0.02-0.03	0.02-0.18
Chicken meat	1.0-1.5	0.7-2.8	93-122	2.6	5.3-9.6	0.11	0.10-0.12
Pork liver	2.7-7.6	29-44	164-223	1.7-5.9	4.0-200	0.90-1.00	1.36-2.21
Fish	0.6-1.7	1.0-3.3	22-84	4.5-9.7	1.2-25	0.02-0.26	0.12
Milk	0.3-0.7	0.2-3.0	0.8-5	0.2-2.0	0.4-4.0	0.01-0.09	0.03-0.28
Cheeses	0,2-0,6	3,3-5,7	0.3-16	0.4-0.8	2.9-4.0	0.02-0.05	0.08-0.82
Eggs	0.7-1.4	2.8-3.5	30	1.9-2.5	16-55	0.09-0.30	0.05-0.80
Flour (wheat)	0.6-5.5	0.2-1.2	9.0-57	1.2-6.0	8.0-13	0.01-0.06	0.60-1.46
Bread	0.6-3.0	0.6-1.5	8.0-34	0.3-3.0	4.0-5.0	0.01-0.02	0.26-0.54
Legumes	2.0-8.4	1.2-2.8	14-31	6.3	9.4-14	0.13-0.60	0.55-1.59
Cabbage	0.5-0.6	0.5	3.0	2.7	1.0-3.0	0.01-0.02	0.16-0.45
Spinach	0.5-1.5	0.6-3.4	6.0	2.2	1.8-27	0.03-0.07	0.50-1.92
Tomatoes	0.6	0.3-0.4	7.0	1.3-1.6	3.0-4.0	0.02-0.04	0.06-0.30
Carrot	0.3-1.4	0.5-2.6	5.0-15	1.0-7.0	3.0	0.03-0.04	0.4
Potatoes	0.5-1.8	0.3-2.0	10-20	1.4-2.3	3.0	0.01-0.02	0.08-0.20
Apples	0.4	0.1	1.0	0.3	1.0	0.01	0,06
Citrus fruits	0.4-1.0	0.2-0.4	1.0-4.0	0.2-1.7	2.0	0.01-0.03	0.05-0.40
Banana	0.5	0.4-0.6	7.0	2.6-3.1	2.0	0.04	0.28-0.36
Nuts	0.5-0.6	0.2-1.3	5.0-9.0	3.0	1.0	0.01-0.91	0.70
Yeast	7.1	17-44	112-200	11-55	50-200	0.80	15

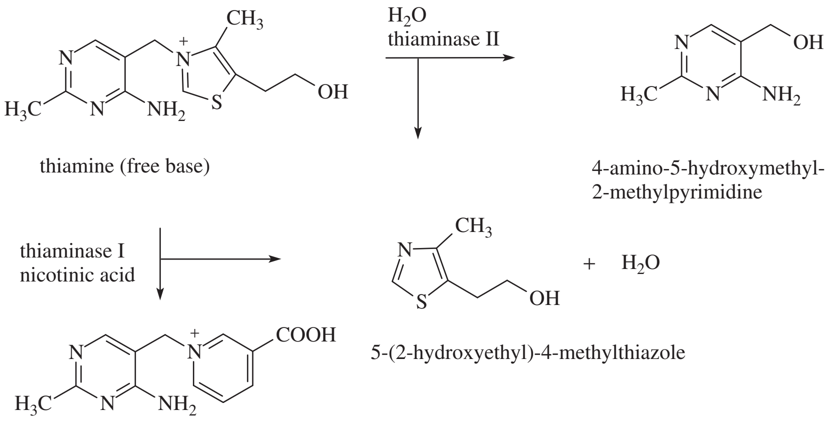
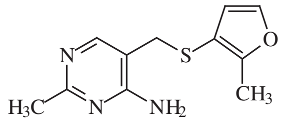


Figure 5.10 Degradation of thiamine by thiaminases.

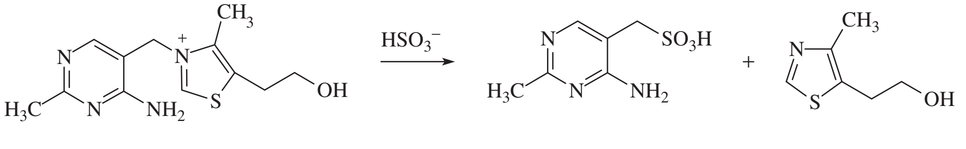
4-methylthiazole and 4-amino-5-aminomethyl-2-methylpyrimidine arises as a minor product. Another non-volatile product, 2-methyl-4-amino-5-(2-methyl-3-furylthiomethyl)pyrimidine (**5-56**), is formed from the pyrimidine moiety of thiamine and a degradation product of thiamine 2-methyl-3-furanthiol. Similarly, the thiamine molecule splits into two parts by the action of sulfur dioxide or hydrogen sulfites (Figure 5.11). This cleavage produces 5-(4-amino-2-methyl-4-pyrimidinyl)methanesulfonic acid and 5-(2-hydroxyethyl)-4-methylthiazole. In aqueous solutions, the thiazole ring of thiamine opens to form thiamine thiol, which exists as a salt in alkaline solutions (both forms also arise in foods). Thiamine thiol is easily oxidised to thiamine disulfide and thiochrome results as a minor product along with some other compounds (Figure 5.12).

A large number of other decomposition products of thiamine arise in aqueous solutions during boiling and by photodegradation.



5-56, 2-methyl-4-amino-5-(2-methyl-3-furylthiomethyl) pyrimidine

More than 70 degradation products have been identified. In addition to simple compounds, such as hydrogen sulfide, ammonia, formaldehyde, acetaldehyde, formic acid and acetic acid, dozens of other minor sulfur compounds are formed: aliphatic sulfides, aliphatic carbonyl compounds with a thiol group, furans containing sulfur in the molecule (including the previously mentioned meaty aroma character imparting compound 2-methyl-3-furanthiol), thiophenes, thiazoles and alicyclic and heterocyclic bicyclic sulfur



thiamine (free base) | 5-(4-amino-2-methylpyrimidinyl)-methane sulfonic acid | 5-(2-hydroxyethyl)-4-methylthiazole

Figure 5.11 Degradation of thiamine by sulfur dioxide.

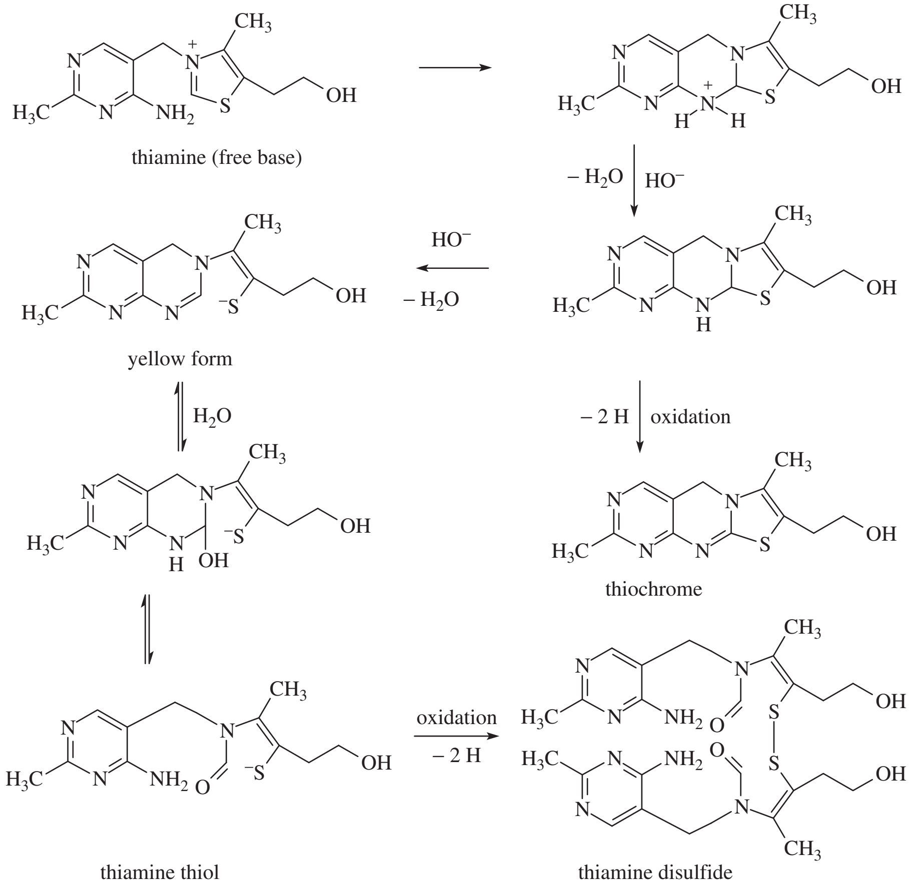


Figure 5.12 Formation of thiamine thiol, thiamine disulfide and thiochrome.

compounds. An important precursor of many decomposition products is 5-hydroxy-3-mercaptopentan-2-one (Figure 5.13). Many of these products become significant flavour components of meat and other foods. Thiamine is therefore often used as a component in mixtures (traditionally mixtures of proteins or amino acids with sugars and other ingredients) called reaction flavours (also known as process flavours) that are powerful, highly concentrated foundations for flavour compounds or seasonings and can be used directly in processed foods. A particularly significant product of thiamine decomposition is bis(2-methyl-3-furyl)disulfide. Its threshold concentrations are extremely low ($2 \cdot 10^{-5}$ μg/kg). This disulfide also appears as a product of thiamine degradation during storage of multivitamin tablets or thiamine tablets, and is responsible for their distinctive odour.

Some reactions of thiamine, such as decarboxylation of α-oxocarboxylic acids, are features of primary metabolism (decarboxylation of pyruvic acid to acetaldehyde in glycolysis and transformation of pyruvic acid to acetyl-CoA prior to its entry into the citric acid cycle) that depend on thiamine diphosphate as a coenzyme. The thiamine ring has an acidic hydrogen and is thus capable of producing the carbanion that acts as a nucleophile towards carbonyl groups. Analogous reactions proceed non-enzymatically and can thus be included among the so-called Maillard reaction. In neutral solutions, for example, thiamine reacts with reducing sugars and other carbonyl compounds (Figure 5.14). Its reaction with glucose yields glucothiamine as a primary product. Reaction with furan-2-carbaldehyde, which is one of the main degradation products of ascorbic acid in acidic solutions, explains the decomposition of thiamine in the presence of vitamin C.

Some amino acids, such as glycine, alanine, valine and glutamic acid, catalyse the thiamine degradation in alkaline solutions. The products are hydrogen sulfide and dethiothiamine (**5-57**). With cysteine and other thiols (and cystine and other disulfides), thiamine reacts to form mixed thiamine disulfides. Such mixed disulfides are the previously mentioned allithiamines (**5-55**). Analogously, thiamine reacts with proteins containing cysteine thiol groups or disulfide bonds in the side chains. These reactions explain the protective effect of thiamine on protein degradation (Figure 5.15).

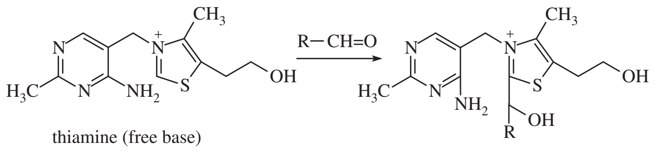


Figure 5.14 Reaction of thiamine with carbonyl compounds.

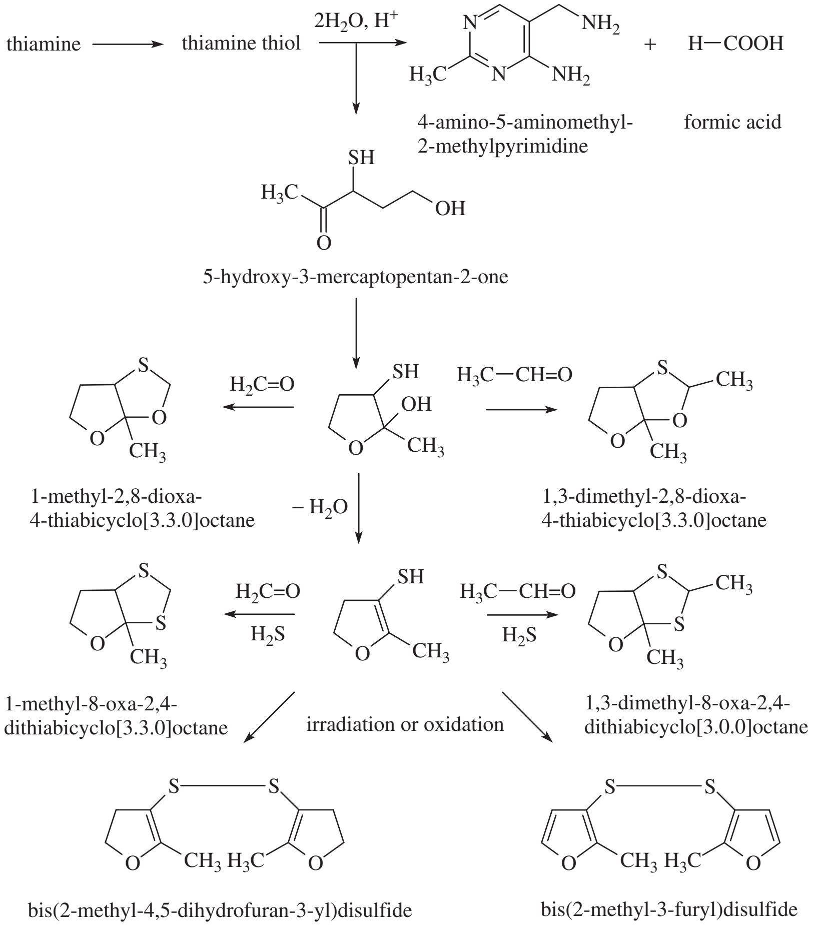


Figure 5.13 Formation of flavour-active products of thiamine.

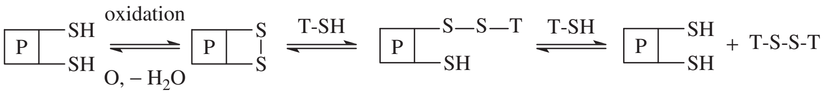
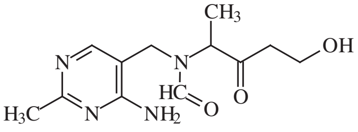


Figure 5.15 Reaction of thiamine with proteins: P = protein, T-SH = thiamine thiol, T-S-S-T = thiamine disulfide.



5-57, dethiothiamine

5.6.7 Changes in foods

5.6.7.1 Meat and meat products

Thiamine decomposes during all food technology and culinary processes, such as frying (by 10–50%), cooking and stewing (50–70%). The losses depend on the size of the processed material, the fat and water content and the method of heat treatment used. The nitrite used in meat curing reacts with thiamine, which is partly decomposed to form elemental sulfur, thiochrome and probably oxythiamine (**5-53**). Freezing and refrigerated storage does not substantially affect the stability of thiamine, but leads to the slow decrease of its content. Frozen meat should be thawed before cooking, but the liquid that drips out during the thawing should be used because it is very rich in thiamine and other nutrients.

5.6.7.2 Milk and dairy products

During pasteurisation, sterilisation (including UHT heating) or drying of milk under normal manufacturing conditions, the loss of thiamine is in the range of 10–20%. The losses are not too high during storage of heat-treated milk either and, of course, are proportional to storage time and temperature. Stability of thiamine in dried milk depends on the conditions during storage, mainly on temperature and the presence of oxygen. Losses usually do not exceed 20%.

5.6.7.3 Cereals and cereal products

Thiamine, like other vitamins, is not evenly distributed in cereal grains. A higher amount of thiamine is found in the outer layers, and the concentration in flour is dependent on the degree of milling (known as flour extraction rate). For example, if the thiamine content of the original wheat is 3.87 mg/kg (expressed on a 13% moisture basis), the thiamine content in the 85% straight run flour (yield of 85%, expressed as a percentage of the wheat represented) is 3.42 mg/kg, in the 80% straight run flour 2.67 mg/kg and in the 70% straight run flour only 0.70 mg/kg, which is 18% of the original amount. Losses during storage of flour are relatively small (usually about 10% according to the conditions and length of storage).

During bread baking, the total losses are relatively small (about 20% of the amount in the flour), but in the crust they reach up to 90%. There are losses of up to 80% in the baking of biscuits. Higher losses result for products where alkaline baking agents (such as carbonates of alkali metals) are used. During grain extrusion, losses are relatively high (20–80%), depending on temperature, water content and the presence of oxygen. Retention of thiamine generally decreases with increased temperature and decreased water content. Cooking of pasta leads to approximately 40% loss of thiamine, which is largely caused by vitamin leakage into the water.

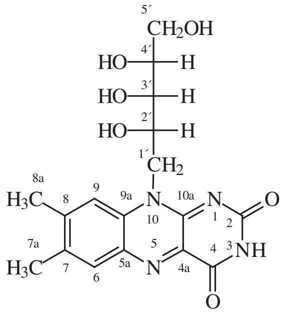
5.6.7.4 Fruits and vegetables

Losses during the cooking of root vegetables are usually around 25% and in leafy vegetables are about 40%. The addition of sulfites, used to prevent enzymatic browning reactions in peeled potatoes or dried fruits and vegetables, results in extensive or total destruction of thiamine.

5.7 Riboflavin

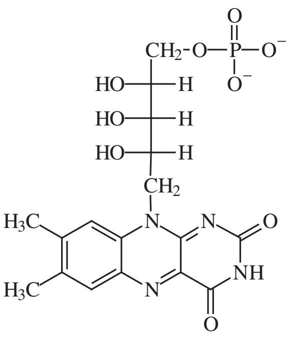
5.7.1 Structure and terminology

The basis of the yellow–green riboflavin structure (vitamin B_2, formerly also known as vitamin G, lactoflavin, ovoflavin or uroflavin) is an isoalloxazine nucleus, to which is bound the alditol ribitol at position *N*-10 (**5-58**). Riboflavin exists in an oxidised (flavoquinone) and a reduced form (flavohydroquinone).

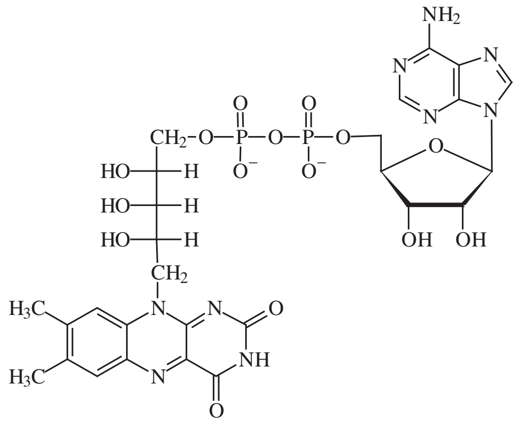


5-58, riboflavin (oxidised form)

Riboflavin, 7,8-dimethyl-10-(1′-D-ribityl)isoalloxazine, likewise occurs as a free compound, but predominantly exists in the form of riboflavin 5′-phosphate (flavin mononucleotide, FMN, **5-59**) and flavin adenine dinucleotide (FAD, **5-60**). These compounds, known as covalently bound riboflavin, are cofactors of enzymes known as flavoproteins, where carbons in positions C-6 or C-8a are involved in covalent bonding of flavins to apoproteins. The corresponding salts are present under physiological pH values. One-electron

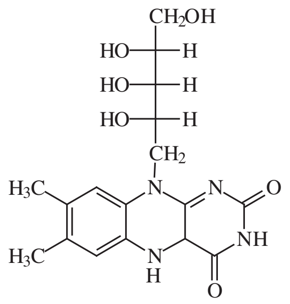


5-59, FMN



5-60, FAD

reduction of riboflavin generates two forms of riboflavin radicals, a red anion and a blue neutral molecule (called flavosemiquinone, Figure 5.16). A reduced, almost colourless form that acts in the enzyme catalysed reduction–oxidation (redox) reactions is 1,5-dihydroriboflavin (another name is dihydroflavin or leucoflavin), which spontaneously oxidises by air oxygen to riboflavin. Another reduced form (generated by two-electron reduction in some enzymatic reactions) is 4*a*,5-dihydroriboflavin (**5-61**).



5-61, 4*a*, 5-dihydroriboflavin

5.7.2 Biochemistry

Riboflavin is synthesised by a variety of microorganisms and plants. The starting substrate is a guanosine 5′-triphosphate (GTP). Animals only transform riboflavin received in food into FMN and FAD. The flavin enzymes are structurally and functionally similar to pteridine enzymes. Flavoproteins containing FMN and FAD cofactors are involved in both one-electron and two-electron redox reactions. One-electron acceptors are haem proteins or proteins containing sulfur and iron; a two-electron donor is, for example, the reduced form of nicotinamide adenine dinucleotide (NADH). Oxygen reacts with the flavins in several ways. For example, it acts as a one-electron acceptor, which yields the superoxide radical or as an acceptor of two electrons, which creates hydrogen peroxide. Most of the enzymes catalysing dehydrogenation, oxygen activation (hydroxylation, monooxygenation) and transmission of electrons are part of the respiratory chain located in the mitochondria. Some enzymes (such as glucose oxidase) are involved in other metabolic processes. In aerobic and some aerotolerant bacteria, riboflavin is involved in the biosynthesis of 5,6-dimethylbenzimidazole, which is a component of vitamin B_{12}.

5.7.3 Physiology and nutrition

The daily requirement of this vitamin is between 0.4 mg (for infants) and 1.7 mg (for adolescents and adult men). In women, the daily requirement is somewhat lower (1.2–1.3 mg), while in pregnant and lactating women it ranges from 1.6 to 1.8 mg or more. It is estimated that nearly 40% of the dietary vitamin comes from milk and dairy products, about 20% from meat and meat products, 15% from cereals, with less than 10% from eggs and the same amount (10%) from vegetables. Riboflavin from foods of animal origin is more easily absorbed in the digestive tract than that from foods of plant origin, where covalently bound forms that are not easily hydrolysed by proteases dominate. Riboflavin deficiency, or ariboflavinose, is relatively rare. It manifests itself mainly in non-specific inflammatory changes (lesions) of the eye, mouth, skin (dermatitis) or mucous membranes. It is usually associated with other vitamin deficiencies such as pellagra.

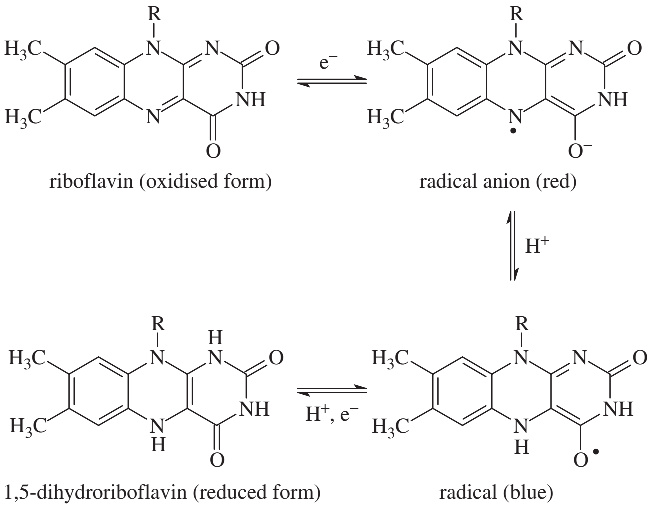


Figure 5.16 Oxidised and reduced forms of riboflavin (see **5-53** for R).

5.7.4 Use

Like thiamine, riboflavin is also used for the fortification of certain foods, such as wheat flour and breakfast cereals. Because of its yellow–orange colour, it is also used as a colouring for certain foods, especially cereal products, ice creams and sugar-coated pills. Hydroxyl groups of ribitol can be easily esterified by carboxylic acids. For example, riboflavin-2′,3′,4′5′-tetrabutyrate has been used as an antioxidant.

5.7.5 Occurrence

5.7.5.1 Foods of animal origin

Riboflavin, mostly as FMN and FAD and less frequently as the free vitamin, is found in all foods, and its distribution is similar to the distribution of thiamine. In milk (and also in eggs), however, the prevailing form is as riboflavin (about 82%). Riboflavin in milk is in part bound to α_S-casein and β-casein, about 14% of riboflavin is in the form of FAD and 4% in the form of FMN. Smaller quantities of some other flavins, such as 10-(2-hydroxyethyl)flavin, 7*a*-hydroxyriboflavin (7-hydroxymethylriboflavin) and its 8*a*-isomer (8-hydroxymethylriboflavin) are also found in milk. A higher vitamin content is found particularly in meat and offal, cheeses and some sea fish (Table 5.8).

5.7.5.2 Foods of plant origin

In addition to riboflavin, FMN and FAD, a large number of other riboflavin derivatives (esters and glycosides) that exhibit similar biological activity, are found in higher plants and microorganisms. For example, the yellow ester of malonic acid (5′-malonylriboflavin) was found in oats (*Avena coleoptiles*, Poaceae) growing in the dark. Riboflavin is present in smaller quantities than thiamine in bread and other cereal products, while whole grain products have a higher riboflavin content.

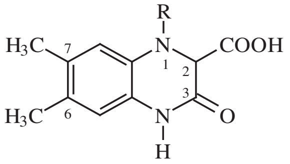
5.7.5.3 Other sources

Unlike thiamine, which remains in the yeast cells during fermentation, a significant amount of riboflavin is present in beer (approximately 0.5 mg/l). Brewer's and baker's yeast is, therefore, a rich source of riboflavin. Derivatives of riboflavin with the C-5′ hydroxyl group of ribitol oxidised to a carbonyl group (called riboflavinal) or a carboxyl group (riboflavinic acid) are present in some edible mushrooms (Basidiomycetes).

5.7.6 Reactions

Flavin coenzymes are very susceptible to enzymatic or chemical hydrolysis. FAD is hydrolysed in acidic solutions to FMN. In acidic solutions, the phosphate group of FMN migrates from the C-5′ position to C′-4, C′-3 and C′-2 positions and subsequent hydrolysis of phosphates yields free riboflavin.

Riboflavin is a very stable vitamin in the absence of light, and in neutral and weakly acidic solutions is practically stable. Even during the production of acid protein hydrolysates (hydrolysis with 20% hydrochloric acid at temperatures above 100 °C), vitamin retention is around 20%. In alkaline media, urea and 1-(3-carboxy-6,7-dimethyl-2-oxo-(2*H*)-quinoxaline-1-yl)-1-deoxy-D-ribitol, also known as 1,2-dihydro-6,7-dimethyl-3-oxo-4-D-ribitol-1-yl-2-chinoxaline carboxylic acid (**5-62**), are formed as reaction products.



5-62, 1,2-dihydro-6, 7-dimethyl-3-oxo-4-D-ribitol-1-yl-2-chinoxaline carboxylic acid (see **5-55** for R)

In neutral and alkaline solutions in the light, all flavins and especially free riboflavin and FMN have complex photochemical properties, act as photosensitisers of type I (free radical generation), type II (oxidation by singlet oxygen) and are degraded to a variety of products (see Section 3.8.1.8.4). Depending on the pH of the solution, excited triplet riboflavin is degraded into non-volatile lumichrome, lumiflavin and other products. The main product of riboflavin photodegradation, after cleavage of the ribitol side chain as 1-deoxy-D-*erythro*-pent-2-ulose (1-deoxy-D-ribulose) in acidic and neutral media, is lumichrome (7,8-dimethylalloxazine). In neutral and alkaline media, the reaction products are lumiflavin, 7,8,10-trimethylisoalloxazine, and D-erythrose (Figure 5.17). Both flavins formed by photodegradation of riboflavin are more effective oxidising agents than riboflavin itself. Flavins also transmit the absorbed light energy to air oxygen (triplet oxygen), which yields singlet oxygen. Singlet oxygen then oxidises flavins, which undergo photolytic fission (photodegradation) and similarly oxidises other organic compounds. For example, the reaction between riboflavin and singlet oxygen yields butane-2,3-dione (biacetyl) (Figure 5.18).

Riboflavin acts as a photosensitisers very often with milk (and also with wine and beer) when exposed to direct sunlight in unsuitable containers (see Section 3.8.1.8.4). Singlet oxygen and

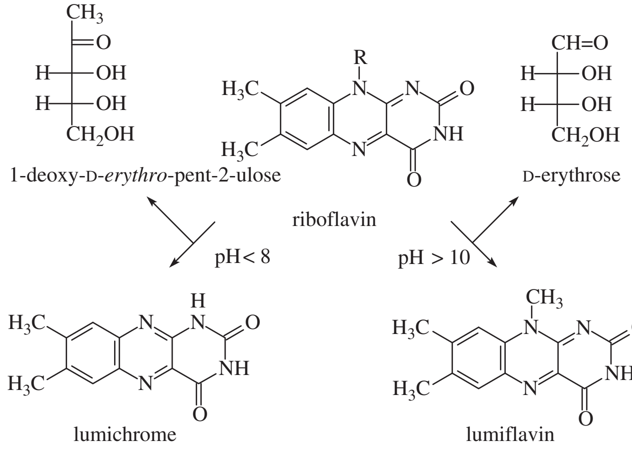


Figure 5.17 Photolysis of riboflavin (see **5-53** for R).

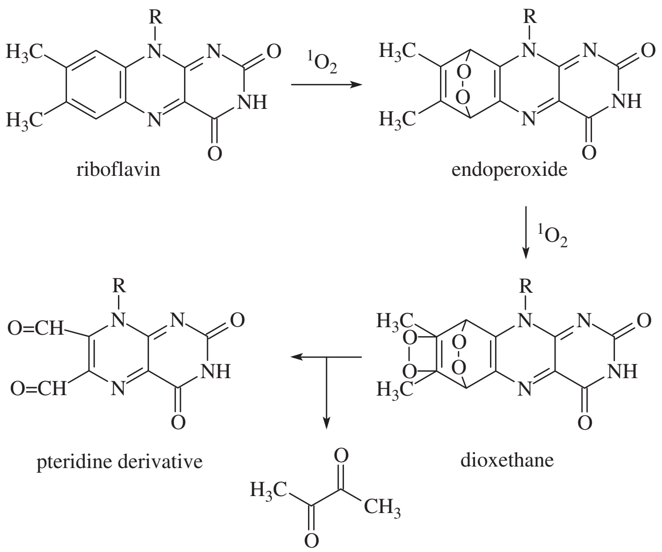


Figure 5.18 Oxidation of riboflavin by singlet oxygen (see **5-53** for R). Jung, Oh, Kim, Kim and Min, 2007, fig 3. Reproduced by permission of the American Chemical Society.

riboflavin degradation products, in particular lumiflavin, cause destruction of vitamin C and oxidise other important substances, such as retinol, essential fatty acids and essential amino acids, and especially methionine.

5.7.7 Changes in foods

Riboflavin is very stable during the thermal processing of foods, but decomposes when exposed to light. To prevent the photochemical degradation of riboflavin, foods containing higher amounts of riboflavin should not be exposed to direct sunlight, and should be stored in opaque or coloured containers.

5.7.7.1 Meat and meat products

Riboflavin is slightly soluble in water (mainly in the reduced form), therefore losses during cooking meat are not very significant (they usually do not exceed 10%), but are caused mainly by extraction into water. During freezing and the refrigerated storage of meat, vitamin loss is also low. For example, the vitamin loss after 15 months of storage of meat is only about 15%.

5.7.7.2 Milk and dairy products

Riboflavin in milk is very stable during normal technological operations (such as pasteurisation and sterilisation); losses do not exceed 5%. For example, retention in UHT milk is about 98%. Losses during storage of UHT milk are also small (about 10%). A significant loss of riboflavin in milk occurs when milk is exposed to direct sunlight. One hour's storage of milk in the sun results in a degradation of about 20–40% of the vitamin present. Diffused daylight also has a similar effect. Riboflavin photosensitisation in milk induces two distinctive off-flavours, which make it less acceptable to consumers. The first off-flavour is the so-called sunlight flavour, giving a burnt and oxidised odour to milk that is caused by methionine oxidation to volatile sulfur compounds, such as methional, methanthiol and dimethyldisulfide. The other off-flavour is a cardboard or metallic flavour, which develops in milk with a prolonged exposure to light. This off-flavour is attributed to some secondary fatty acid oxidation products that include aldehydes, especially (*E*)-non-2-enal, ketones, alcohols, hydrocarbons and other compounds. Fermented milk products contain, in most cases, higher concentrations of riboflavin than the original milk, because the vitamin is synthesised by the microorganisms used. During the drying of milk, about 2% of riboflavin is lost.

5.7.7.3 Cereals and cereal products

Cereals contain a considerable amount of riboflavin. The vitamin content in flour depends, as with other water-soluble vitamins, on the degree of milling (flour extraction rate). Its content is higher in dark flours than in white flours. Losses during cooking are also small (up to 10%); higher losses (up to 30%) are found in flours fortified with riboflavin. Losses in cooked pasta products (to a large extent caused by leaching) reach, according to the type of pasta, 35–55%.

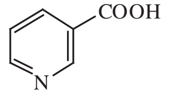
5.7.7.4 Fruits and vegetables

Riboflavin losses in canned fruits and vegetables are in the range of 25–70%, according to the type of processed material. The main losses are caused by leaching. For example, cooked vegetables lose 30–40% of their riboflavin content by leaching into the water used for cooking.

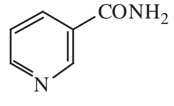
5.8 Niacin

5.8.1 Structure and terminology

Niacin, also formerly known as PP factor (Pellagra Preventive factor) or vitamin PP is a common name for nicotinic acid (pyridine-3-carboxylic acid, **5-63**) and its amide nicotinamide (niacin amide, also formerly known as vitamin B_3, **5-64**). Both compounds have the same biological activity.



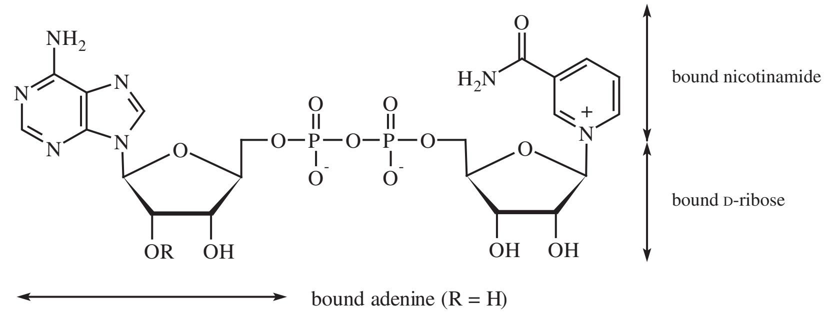
5-63, nicotinic acid



5-64, nicotinamide

5.8.2 Biochemistry

Nicotinamide is part of nicotinamide adenine dinucleotide, NAD (oxidised form is NAD^+ and reduced form is NADH) and its phosphoric acid ester nicotinamide adenine dinucleotide phosphate, NADP ($NADP^+$ and NADPH, **5-65**), which are



5-65, NAD^+, R = H
$NADP^+$, R = PO_3H_2

cofactors (coenzymes) of several hundreds of different enzymes. In the older literature, these substances are known as diphosphopyridine nucleotide (DPN^+, DPNH) and as triphosphopyridine nucleotide (TPN^+, TPNH).

Both cofactors are involved in respiratory electron transfer systems (Figure 5.19), for example, in most redox reactions of the citric acid (Krebs) cycle. NAD is most often involved in degradation (catabolism) of sugars, fats, proteins and ethanol, while NADP is involved mainly in biosynthetic (anabolic) reactions, such as synthesis of macromolecules, fatty acids and cholesterol. Dinucleotides, in addition of their activities in redox reactions, participate in post-translational modifications of some proteins and other reactions.

$NAD(P)^+$ from the diet is first hydrolysed to a mixture of nicotinic acid and nicotinamide. Nicotinic acid can then be transformed into nicotinamide and then to $NAD(P)^+$ in the body. These dinucleotides are *de novo* synthesised by bacteria and some plants from aspartic acid and 1,3-dihydroxyacetone phosphate (glycerone phosphate). Quinolinic acid is an intermediate. It arises from tryptophan in some microorganisms and in animals.

Biosynthesis and catabolism of $NAD(P)^+$ produce various derivatives of niacin, such as N^1-methylnicotinamide (a product of catabolism in humans and animals) and *N*-methylnicotinic acid (1-methylpyridinium-3-carboxylate), known as trigonelline (Figure 5.20), which is a product of catabolism in plants and fungi. Previously, trigonelline was known under the names caffearin, coffearin or gynesine. Trigonelline was first found in fenugreek seeds (*Trigonella foenum-graecum*, Fabaceae)[7] and subsequently in many other plant species, such as legumes, cereals and potatoes. Trigonelline is a biologically active compound involved in the induction of leaf movement, it acts as an osmoprotectant and probably serves as a reserve source of nicotinic acid.

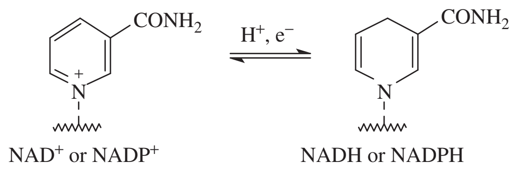


Figure 5.19 Oxidation of NADH and NADPH and reduction of NAD^+ and $NADP^+$.

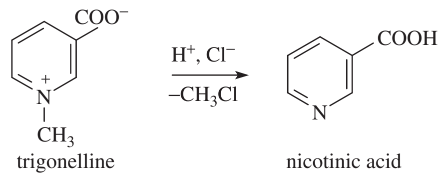


Figure 5.20 Pyrolysis of trigonelline.

5.8.3 Physiology and nutrition

The need for niacin is not precisely known, since it depends on many factors, such as the amount of protein (tryptophan and leucine) consumed. Leucine is an inhibitor of NAD biosynthesis from tryptophan. The recommended daily dose for children is 2–12 mg, 14 mg for women, 16 mg for men and 18 mg for pregnant and lactating women.

The niacin requirement is mainly provided by meat and meat products (about 40% or more), milk (about 10%), cereal products (about 20%), potatoes (about 10%) and other foods. Meat and meat products supply about 50% of the needs for tryptophan, milk and eggs about 25%. Lack of the vitamin, known as pellagra, is manifested mainly by skin damage, digestive tract disorders and later by mental disorders (dementia).

Man has a significant but limited ability to synthesise niacin in a somewhat complicated way using tryptophan and enzymes containing vitamin B_6 as a cofactor. It is reported that 34–86 mg (mean 60 mg) of tryptophan is required for biosynthesis of 1 mg of niacin. The possibility of biosynthesising niacin from tryptophan explains the beneficial effect of milk and eggs as a protection against pellagra. Both foods are good sources of tryptophan, although they contain low concentrations of niacin.

Some pyridine derivatives act as antagonists of niacin. Antagonists occurring in foods may include 3- and 4-acetylpyridine that arise as products of the Maillard reaction. *N*-Methylpyridinium cation generated by the thermal degradation of trigonelline inhibits gastric acid secretion and induces phase II detoxifying enzymes.

[7]The plant comes from the Mediterranean region and is now cultivated worldwide and used as a spice (it is a common ingredient of many curries and other spice mixtures), medicinal plant and animal feed. For example, mixed with summer savoury (*Satureja hortensis*) of the mint family (Lamiaceae), fenugreek seed is a component of chubrica, a favourite spice of Bulgarian cuisine.

5.8.4 Use

In some countries, nicotinic acid is added to white wheat flour and to other cereal products. The complex with menadione bisulfite, which shows the activity of niacin and vitamin K, has been used in animal nutrition. Nicotinamide in combination with ascorbic acid is used as a meat colour stabiliser, but has not found wider application in the meat industry (under anaerobic conditions, metmyoglobin in raw meat is reduced back to myoglobin by enzymes that contain NAD as a cofactor).

5.8.5 Occurrence

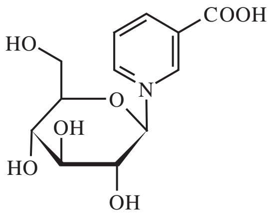
Distribution of niacin in foods is similar to other B group vitamins (Table 5.8). Niacin is present in small amounts in all foods, usually in a bound form.

5.8.5.1 Foods of animal origin

Foods of animal origin mainly contain nicotinamide, mostly in the form of NAD and NADP. The richest source is offal, meat and meat products, eggs and especially egg yolk (which contains approximately 60 mg/kg of niacin). Milk contains a surprisingly low amount of niacin; the niacin content of cheeses is higher.

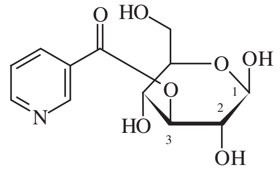
5.8.5.2 Foods of plant origin

The main form of this vitamin in foods of plant origin is nicotinic acid. Plants contain a number of nicotinic acid derivatives, such as *N*-(β-D-glucopyranosyl)nicotinic acid (**5-66**), the ester 1-*O*-nicotinoyl-β-D-glucopyranose and other nicotinoyl derivatives of glucose.



5-66, *N*-(*β*-D-glucopyranosyl)nicotinic acid

Cereals often have, at first sight, a considerable niacin content. Wheat contains about 30–70 mg/kg of niacin, and whole wheat flour and brown rice contain about 30–60 mg/kg. However, milling and peeling have a great influence on the vitamin content, because niacin is largely located in the germ and bran. For example, wheat grains containing niacin at a level of 57 mg/kg give 80% and 70% straight-run flours that have a vitamin content of 19 and 10 mg/kg, respectively. The same vitamin level (about 10 mg/kg) is found in white bread and white rice. Nicotinic acid in maize and sorghum grains is largely covalently bound to macromolecular glycopeptides and is generally not available to mammals. Partial hydrolysis of these glycopeptides gives 3-*O*-nicotinoyl-β-D-glucopyranose (**5-67**). The lack of bioavailability of nicotinic acid in maize, which leads to pellagra, is probably due to this type of binding. The biological availability of nicotinic acid in maize is significantly improved by pre-treatment of the maize flour with lime milk, a procedure used in Mexico for the preparation of tortillas. For example, the total amount of the vitamin in raw maize grains is about 30 mg/kg, of which less than 2% are bioavailable. Boiling in water increases the vitamin utilisation to 16%, but when cooked in lime milk, vitamin utilisation is 100%. Legumes, fruits, vegetables and potatoes have average amounts of niacin.



5-67, 3-*O*-nicotinoyl-β-D-glucopyranose

5.8.5.3 Other sources

A surprisingly rich source of niacin is roasted coffee. Green coffee beans contain a large amount of the alkaloid trigonelline, which is demethylated on roasting forming nicotinic acid (Figure 5.20). The content of niacin in roasted coffee is about 25 times higher than in green (unroasted) coffee and reaches up to 2% of dry matter. Together with other compounds, it also contributes to the bitter taste of roasted coffee. Decarboxylation of trigonelline gives a small amount of the *N*-methylpyridinium cation, transmethylation yields methyl nicotinate and other reactions yield a number of volatile flavour-active pyrroles and pyridines. The caffeine content is virtually unchanged during roasting, so the ratio of caffeine to trigonelline serves as a criterion of the degree of coffee roasting.

5.8.6 Reactions

Nicotinic acid is very stable when heated in aqueous solutions, and is stable in acidic and alkaline media. Nicotinamide is very stable in neutral solutions, in acidic and alkaline solutions it is hydrolysed to nicotinic acid.

5.8.7 Changes in foods

5.8.7.1 Meat and meat products

When processing raw meat and offal, the content of free nicotinamide increases due to the enzymatic hydrolysis of NAD and NADP. Vitamin losses during the thermal processing of meat usually do not exceed 10%, while losses due to draining from improper thawing can amount to 50%. Partial hydrolysis of nicotinamide to nicotinic acid may also occur.

5.8.7.2 Milk and dairy products

Losses in milk are very small (up to 5%). In the manufacture of cheeses, the majority of the niacin goes into the whey (the liquid

part of milk that remains after the manufacture of cheeses). Losses during maturation and storage of cheeses are negligible. The niacin content of yoghurts is slightly higher than in milk because it is produced by the microorganisms that are present.

5.8.7.3 Cereals and cereal products

The loss of the vitamin does not usually exceed 10% in baked cereal products. The use of alkalising baking ingredients, such as baking powders based on ammonium bicarbonate, can increase the bioavailability of niacin due to its release from unavailable forms.

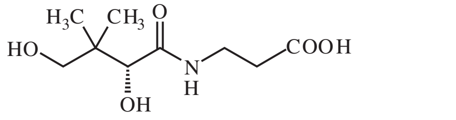
5.8.7.4 Fruits and vegetables

Typically, losses of niacin in preserved fruits and vegetables and in cooked potatoes do not exceed 30–40%; the main reason for any loss is leakage into water.

5.9 Pantothenic acid

5.9.1 Structure and terminology

Pantothenic acid (formerly also known as vitamin B_5, **5-68**) occurs in nature only as the (+)-D-form, that is the (*R*)-enantiomer. (*R*)-Pantothenic acid is composed of (+)-D-pantoic acid, also known as (*R*)-pantoic acid, the systematic name of which is (*R*)-2,4-dihydroxy-3,3-dimethylbutanoic acid. Pantoic acid is linked by an amide bond to the 3-aminopropionic acid (β-alanine). The (*S*)-enantiomer of pantothenic acid, which is the (−)-L-isomer, is not biologically active and is an antimetabolite of (*R*)-pantothenic acid, as are some other structural analogues. The main biologically active natural form of pantothenic acid is coenzyme A (CoA, HS-CoA or CoASH, **5-69**)[8] and a protein called acyl-carrier protein (ACP, **5-70**).



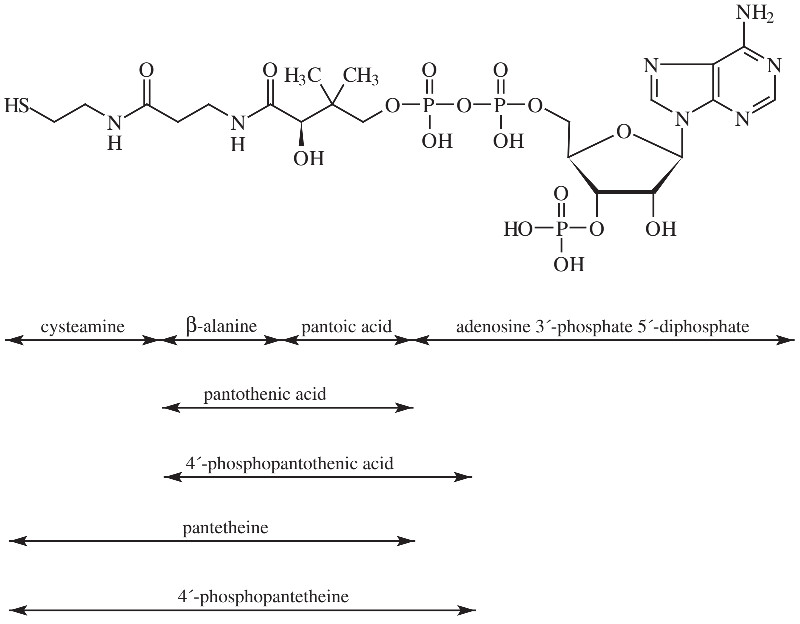
5-68, (*R*)-pantothenic acid

Pantothenic acid can be accompanied by its biologically active higher homologue known as homopantothenic acid (**5-71**), which contains 4-aminobutanoic acid (γ-aminobutyric acid) instead of β-alanine and acts as an antagonist of pantothenic acid.

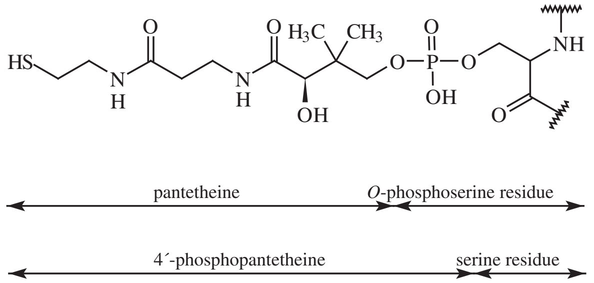
5.9.2 Biochemistry

Pantothenic acid is an essential nutritional factor for a range of yeasts, lactic acid and propionic acid bacteria and other microorganisms. Some bacteria and plants synthesise this acid *de novo* from pantoic acid and β-alanine. The biosynthesis of pantoic acid uses 3-methyl-2-oxobutanoic (2-oxoisovaleric) acid, which is a precursor of valine, the donor of the hydroxymethyl group is 5,10-methylenetetrahydrofolic acid, and decarboxylation of aspartic acid yields the β-alanine.

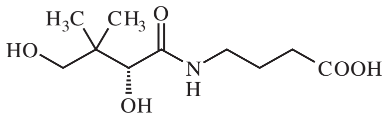
[8]Coenzyme A is the active component of transacylases transporting the residues of carboxylic acids. The most common substance is acetyl-CoA, which carries acetyl groups. In acetyl-CoA, acetic acid is bound as a thioester to the thiol group of cysteamine. Other acyl coenzymes A include malonyl-CoA, succinyl-CoA, and other coenzymes that play a role in the metabolism of proteins, fats and sugars. ACP plays a fundamental role in biosynthesis of fatty acids and polyketides.



5-69, coenzyme A

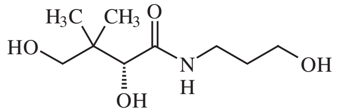


5-70, ACP



5-71, (*R*)-homopantothenic acid

Animals (and also some yeasts and bacteria) do not synthesise pantothenic acid as they only convert exogenous vitamin obtained from food into both metabolically active forms, coenzyme A and ACP. Exogenous coenzyme A is first hydrolysed to pantothenic acid and pantetheine (via 4′-phosphopantetheine); exogenous (*R*)-panthenol (**5-72**) is oxidised to pantothenic acid.



5-72, (*R*)-panthenol

5.9.3 Physiology and nutrition

The recommended daily intake of pantothenic acid in infants under 6 months of age is 1.7 mg, and is 2 mg in children from 6 to 12 months, 3 mg in children from 4 to 8 years, 4 mg in children from 9 to 13.5 years, 5 mg in adolescents and men and 6 mg in women (7 mg in lactating women). Normal dietary intake of this vitamin is 6–12 mg per day. Cases of deficiency, which us manifested mainly by skin irritation and paraesthesia (a tingling and itching sensation), are not common.

It is known that homopantothenic acid improves the metabolism of glucose in the brain and the higher functions of the brain and it has been used in Japan to enhance mental functions, especially in Alzheimer's disease. A rare side-effect is an abnormal brain function resulting from the failure of the liver to eliminate toxins (hepatic encephalopathy). This condition was reversed by pantothenic acid supplementation, suggesting that it was due to pantothenic acid deficiency caused by the antagonist homopantothenic acid.

5.9.4 Use

Pantothenic acid or its salts are added to foods only occasionally. Calcium and sodium pantothenate are more stable and less hygroscopic than the free acid. Salts of pantothenic acid and less polar (*R*)-pantothenyl alcohol or (*R*)-panthenol (**5-72**) are used to fortify animal feed for farm animals (mainly poultry) and also in pharmacy and cosmetics. Proportionally, owing to increasing consumption of cooked foods and ready-to-eat meals in developed countries, more extensive fortification of foods can be expected.

5.9.5 Occurrence

Pantothenic acid is found in virtually all foods of plant and animal origin, usually in relatively small amountss (Table 5.8). Only a small proportion of the total vitamin content is free acid; bound forms such as coenzyme A, acyl-coenzymes A and ACP are mostly present. The pantothenic acid levels in individual foods is highly variable.

5.9.5.1 Foods of animal origin

Relatively large amounts of the vitamin occur in meat, and particularly in offal, eggs and some types of cheese. There is a low vitamin content in milk.

5.9.5.2 Food of plant origin

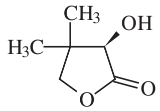
The pantothenic acid content in wheat flour is strongly influenced by the degree of milling. There is a higher vitamin level in wholegrain flour, cereal products and legumes, whilst about ten times lower vitamin content is typically found in fruits and vegetables. Homopantothenic acid occurs in cooked rice at concentrations of about 20 mg/kg.

5.9.5.3 Other sources

Similarly to the other B group vitamins, relatively large amounts of pantothenic acid are found in yeasts. Homopantothenic acid occurs in dried yeasts in a concentration of 8500 mg/kg.

5.9.6 Reactions

The stability of pantothenic acid in aqueous solutions depends greatly on the pH value. The vitamin is most stable in weakly acidic (pH 4–5) solutions, but in acidic and alkaline media the amide linkage is hydrolysed and pantothenic acid yields pantoic acid (or its salt) and β-alanine. The enzyme pantothenase of some bacteria specifically cleaves pantothenic acid into the same products. In acidic solutions, pantoic acid spontaneously dehydrates to form lactone, (*R*)-2-hydroxy-3,3-dimethylbutano-4-lactone, which is called pantoyllactone or pantolactone (**5-73**). Analogously, products of panthenol hydrolysis are pantoic acid



5-73, (*R*)-pantolactone

and 3-aminopropane-1-ol (β-alanol). Solutions of coenzyme A and pantetheine are relatively stable in solutions of pH 2–6. Both compounds are easily oxidised to form disulfides, pantethine or the disulfide form of coenzyme A, respectively.

5.9.7 Changes in foods

Pantothenic acid is relatively unstable during storage and especially during the thermal processing of foods. Losses caused by leaching into the water during operations such as washing, blanching and cooking are often higher than the losses caused by hydrolysis.

5.9.7.1 Meat and meat products

Thermal processing of meat results in losses of pantothenic acid that reach about 12–50%, depending on the conditions. The extent of these losses is contingent on the type of heat treatment, the volume of water used and other factors. Losses in canned meat and in meat products range from 20 to 35%.

5.9.7.2 Milk and dairy products

Pasteurisation of milk does not significantly affect the vitamin content. In the case of UHT milk, the vitamin loss is about 5% of the original level and the loss after 6 weeks of storage of UHT milk is 20–35%. The natural vitamin content in fermented dairy products is affected only slightly by fermentation. Milk powder manufacture and storage of milk powder result in small losses of the vitamin.

5.9.7.3 Cereals and cereal products

A small decrease in pantothenic acid concentration occurs during milling. The vitamin retention in bread is relatively high (up to 90%), while in cooked pasta, according to the type of product and method of preparation, it reaches 55–75%. Losses during the cooking of legumes are 25–56% depending on soaking time. Higher amounts of pantothenic acid can be lost when exposed to alkali agents, such as baking soda.

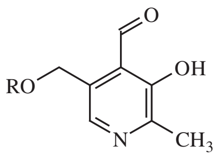
5.9.7.4 Fruits and vegetables

In preserved fruits and fruit juices, the losses of pantothenic acid are on average about 50%. In preserved vegetables, where the vitamin is exposed to vinegar, the losses can reach 45–80%.

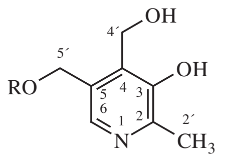
5.10 Pyridoxal, pyridoxol and pyridoxamine

5.10.1 Structure and terminology

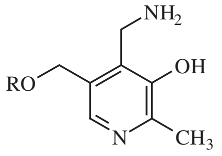
The name vitamin B_6 (formerly known as adermine) refers to three structurally related, biologically active derivatives of 3-hydroxy-5-hydroxymethyl-2-methylpyridine and to their 5′-phosphates. These three forms of vitamin B_6 differ in their substitution at position C-4 of the pyridine ring: the formyl derivative, 4-formyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine, is called pyridoxal (**5-74**); the hydroxymethyl derivative, 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine, is called pyridoxol or pyridoxine (**5-75**); and the aminomethyl derivative, 4-aminomethyl-3-hydroxy-5-hydroxymethyl-2-methylpyridine, is called pyridoxamine (**5-76**). The residue that results after splitting off the 4′-hydroxyl group is known as the pyridoxyl residue, the pyridoxylidene residue forms by splitting off the oxygen from the aldehyde group of pyridoxal.



5-74, pyridoxal, R = H
pyridoxal 5′-phosphate, R = PO_3H_2



5-75, pyridoxol, R = H
pyridoxol 5′-phosphate, R = PO_3H_2



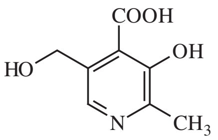
5-76, pyridoxamine, R = H
pyridoxamine 5′-phosphate, R = PO_3H_2

5.10.2 Biochemistry

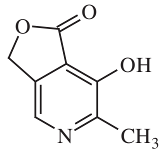
Prokaryotic organisms synthesise a primary form of vitamin pyridoxol 5′-phosphate from 1-deoxy-D-xylulose 5-phosphate (1-deoxy-D-*threo*-pent-2-ulose 5-phosphate) and 2-amino-2-deoxy-D-*threo*-tetronic (2-amino-2-deoxy-D-threonic) acid, known as 4-(phosphohydroxy)-L-threonine or 4-hydroxy-L-threonine 4-phosphate. Phosphohydroxythreonine arises from D-erythrose 4-phosphate, a product of decomposition of D-fructose 6-phosphate. Non-phosphorylated forms (pyridoxal, pyridoxol and pyridoxamine) are produced by hydrolysis of the corresponding phosphates. Animals do not synthesise vitamin B_6 *de novo*, only convert the non-phosphorylated forms in the liver, erythrocytes, and other tissues into the corresponding phosphates and the individual forms of each other. Pyridoxal 5′-phosphate arises by oxidation of pyridoxol 5′-phosphate and transamination of pyridoxal 5′-phosphate provides pyridoxamine 5′-phosphate. Both these forms of vitamin B_6 are catalytically active. Pyridoxal 5′-phosphate

(originally called codecarboxylase) is a cofactor for enzymes catalysing decarboxylation, deamination, racemisation, transamination and transsulfuration of amino acids and also participates in many reactions related to metabolism of fats and sugars.

The dominant forms in animal tissues are pyridoxal 5′-phosphate and pyridoxamine 5′-phosphate. An important metabolite of pyridoxal excreted in the urine is biologically inactive 4-pyridoxic acid (**5-77**) and its lactone, which is called 4-pyridoxolactone (**5-78**). Minor metabolites generated by oxidation in position C-5′are isopyridoxal (aldehyde), the corresponding carboxylic acid and its lactone. Pyridoxol 5′-phosphate is the dominant form in plant tissues.



5-77, 4-pyridoxic acid

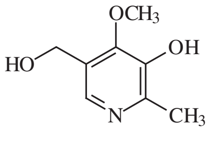


5-78, 4-pyridoxolactone

5.10.3 Physiology and nutrition

The recommended daily intake of vitamin B_6 is 0.3–2.6 mg (the lower limit is for babies and the upper limit for pregnant and lactating women). Vitamin B_6 deficiency manifests by dermatitis and neurological disorders (seizures in children). Long-term high-dose vitamin intake can cause neurological disorders, manifesting as loss of sensation in the feet and poor coordination. Such increased vitamin intake can be only provided by vitamin supplements. It is estimated that about 40% of the required vitamin is provided by meat and meat products, 22% by vegetables, 12% by milk and dairy products, 10% by cereals, 5% by legumes, 8% by fruits and 2% by eggs.

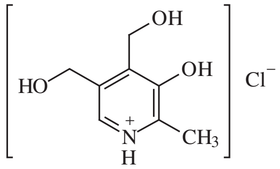
Vitamin antagonists are compounds reacting with the carbonyl group of pyridoxal or substances structurally related. Natural antagonists may be some tryptophan metabolites, hydrazines and hydroxylamines that react to form the respective hydrazones and oximes, which are unavailable forms of vitamin B_6. Some reaction products of pyridoxal with amino acids are also unavailable or available to a small extent. Vitamin antagonists include linatin, an amino acid present in flax seeds and a neurotoxin gingkotoxin (4′-*O*-methylpyridoxol, **5-79**), which is a minor component of gingko leaves (*Ginkgo biloba*, Ginkgoaceae), known as the maidenhair tree.



5-79, gingkotoxin

5.10.4 Use

For the fortification of foods and for food supplements, synthetic pyridoxol hydrochloride (**5-80**) is used. Baby food, and in some countries white wheat flour, are the foods most often fortified.



5-80, pyridoxol hydrochloride

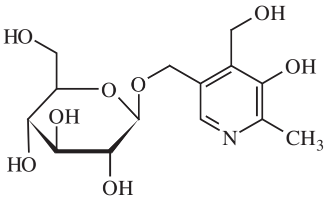
5.10.5 Occurrence

5.10.5.1 Foods of animal origin

Meat, meat products, offal and egg yolk are rich sources of vitamin B_6 (Table 5.8). In foods of animal origin, the main compounds are pyridoxal and pyridoxamine, especially in the form of phosphate esters. For example, in meat the main form is present as pyridoxal 5′-phosphate (about two thirds of this vitamin are prosthetic groups of enzymes) bound to various proteins (such as imine), to a lesser extent as free pyridoxal 5′-phosphate, followed by pyridoxamine 5′-phosphate. In contrast, milk contains only about 10% of vitamin in bound forms. The vitamin content in milk and cheeses is relatively low.

5.10.5.2 Foods of plant origin

Foods of plant origin mainly contain pyridoxol and pyridoxal. Glycosides of pyridoxol are also common. The predominant form is 5′-*O*-(β-D-glucopyranosyl)pyridoxol (**5-81**), which in fruits and vegetables represents 5–80% of the total vitamin content. This form is less available than free pyridoxal. A minor component of pea (*Pisum sativum*, Viciaceae) is 5′-*O*-(β-D-glucopyranosyl)pyridoxol esterified by 3-hydroxy-3-methylglutaric acid at position C-6 of D-glucose. Cereals are good sources of vitamin B_6. A higher vitamin content can be found in whole grain cereal products and cereal germs, as well as in some vegetables, potatoes and legumes.



5-81, 5′-*O*-(β-D-glucopyranosyl)pyridoxal

5.10.5.3 Other sources

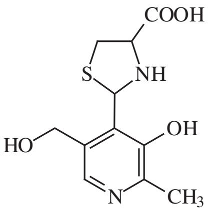
Vitamin B_6 is, like the other B vitamins, present in high concentrations in yeast.

5.10.6 Reactions

Vitamin B_6 is relatively stable in acidic solutions, and less stable in neutral and alkaline solutions, particularly in the light. Pyridoxol is more stable than pyridoxal and pyridoxamine.

Amino acids, peptides and proteins react with pyridoxal and its phosphate in neutral solutions to form imines (formerly known as Schiff bases). This reaction is analogous to reactions of reducing sugars in the Maillard reaction (Figure 5.21). Pyridoxal 5′-phosphate is more reactive than free pyridoxal. Reaction products with amino acids and proteins (imines) isomerise and are hydrolysed to pyridoxamine and the corresponding 2-oxocarboxylic acids (non-enzymatic transamination reaction). The addition of the second amino acid molecule (or thiol) to imines yields *N*,*N*′-substituted diamines. For example, reduction of imines by ascorbic acid or reductones arising in the Maillard reaction yields stable pyridoxylamines (*N*-substituted pyridoxamines). Imines and diamines formed from imines and amino acids are physiologically fully available, pyridoxylamino acids are very resistant to acid hydrolysis and, therefore, less physiologically active. Reaction of pyridoxal 5′-phosphate with proteins generates about 20% of bound forms (8% imines and diamines and 8% of pyridoxylamino acids).

Amino acids containing other reactive functional groups react with pyridoxal to form heterocyclic compounds. Reaction with cysteine during sterilisation of milk gives a thiazolidine derivative (**5-82**). Reaction with tryptophan gives a β-carboline derivative (**5-83**). On a small scale, the Strecker degradation of amino acids yields the corresponding Strecker aldehydes. Hydrogen sulfide produced by the degradation of cysteine reacts with the carbonyl group of pyridoxal analogously to the amino group of amino acid or protein, and an unstable mercaptal forms as an intermediate. It is a precursor of pyridoxylthiol and bis(4-pyridoxyl)disulfide. Reaction with protein-bound cysteine yields mixed disulfides (Figure 5.22). Pyridoxylthiol and the corresponding disulfide show only low activity of vitamin B_6.



5-82, thiazolidine derivative of pyridoxal

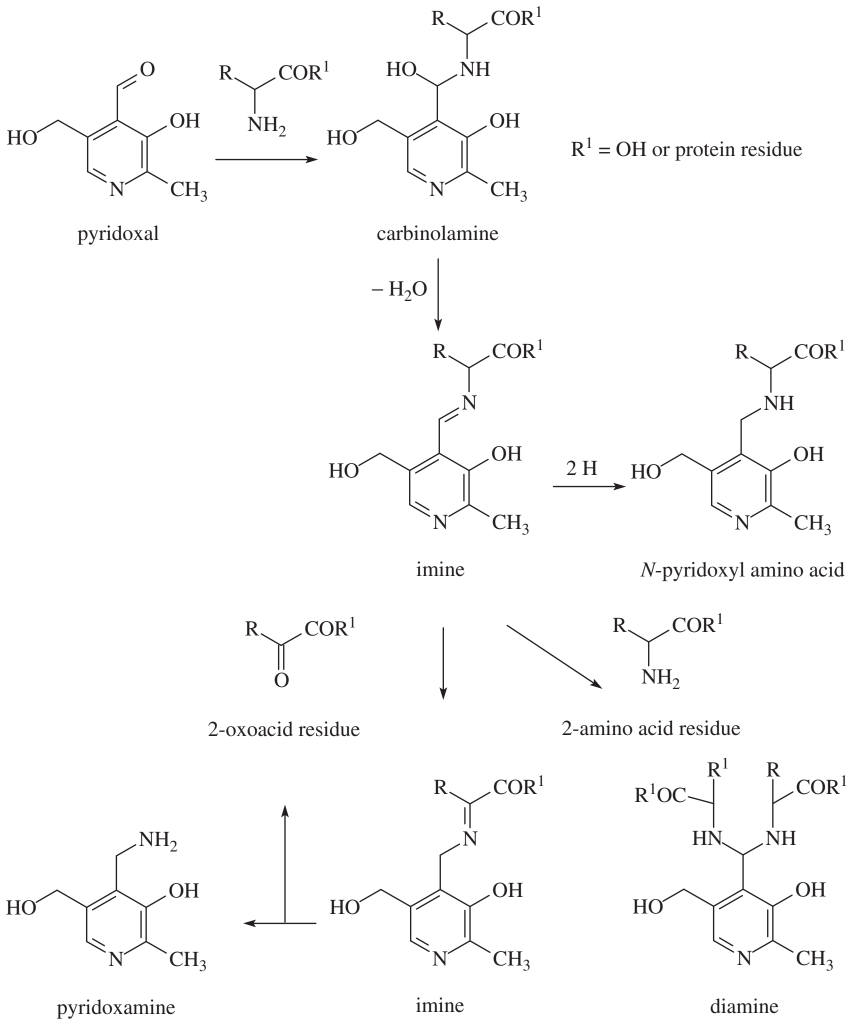
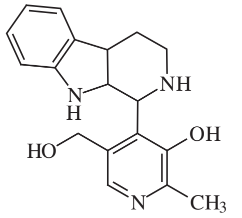


Figure 5.21 Reactions of pyridoxal with amino acids (R^1 = OH) and proteins (R^1 = NHR^2).



5-83, β-carboline derivative of pyridoxal

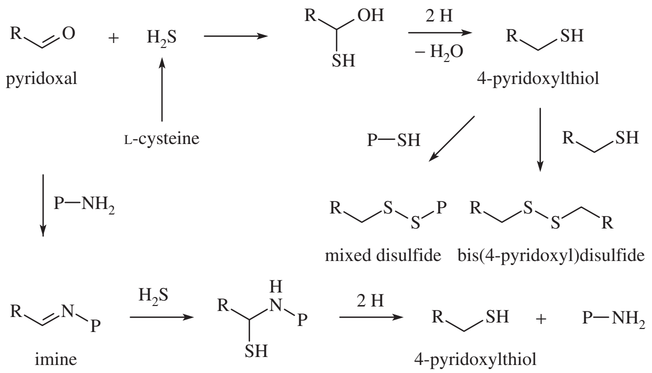


Figure 5.22 Reaction of pyridoxal with thiol group of cysteine and proteins (P = protein residue).

5.10.7 Changes in foods

Vitamin losses during food storage and processing vary considerably in different commodities according to the predominant form of the vitamin. In foods of plant origin that contain less volatile pyridoxol, vitamin losses are usually small. In foods of animal origin containing reactive pyridoxal, losses are higher. Losses are also attributed to vitamin leaching and pyridoxal reaction with proteins.

5.10.7.1 Meat and meat products

Raw meat contains pyridoxal phosphate as the main vitamin form, whereas cooked meat predominantly contains a product of its transamination pyridoxamine phosphate. For example, the main form of vitamin B_6 in raw chicken meat is pyridoxal phosphate (56%), followed by pyridoxamine phosphate (42%) and pyridoxamine (2%). The main form in roasted chicken meat is pyridoxamine phosphate (70%), followed by pyridoxal phosphate (21%), pyridoxol (7%) and pyridoxamine (2%). Vitamin retention in roasted meat is about 45–65%.

5.10.7.2 Milk and dairy products

During conventional methods of milk processing, vitamin losses are small. For example, during pasteurisation the loss is up to 5%, while loss in UHT milk does not exceed 10%. Higher losses are caused by subsequent storage, so that the total losses in UHT milk are about 40–45%. During the heat treatment of milk, pyridoxal is partly transformed into pyridoxamine. Fresh milk mainly contains pyridoxic acid (38%), pyridoxal phosphate (32%), pyridoxal (24%), pyridoxamine (4%) and pyridoxamine phosphate (2%). In pasteurised milk, the main form is pyridoxal (41%), followed by pyridoxal phosphate (24%), pyridoxic acid (20%), pyridoxamine phosphate (9%) and pyridoxamine (6%).

During pasteurisation and especially during drying, pyridoxal reacts with cysteine and lysine (free amino acids and amino acids bound in proteins). For example, milk powder contains 30–70% of the original amount of vitamin. The losses are higher when milk is exposed to sunlight.

5.10.7.3 Cereals and cereal products

The main form of vitamin B_6 in cereals is free pyridoxol and pyridoxol bound to glucose. The vitamin content of flour depends on the degree of milling. In dough and also in bread, the losses are small and do not exceed 15%. Vitamin retention in cooked pasta is 50–70%.

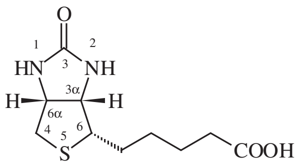
5.10.7.4 Fruits and vegetables

Relatively large losses occur during canning and cooking of vegetables (40–50%), while fruit losses are somewhat lower (approximately 40%).

5.11 Biotin

5.11.1 Structure and terminology

Biotin is composed of an ureido (tetrahydroimidizalone) ring fused with a tetrahydrothiophene ring substituted with pentanoic (valeric) acid. The biotin molecule contains three asymmetric carbon atoms. Only one of eight possible isomers, that is (3*aS*,4*S*,6*aR*)-5-[2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoic acid, known as *d*-biotin or (+)-biotin (**5-84**), occurs in nature and is biologically active. Biotin was also known as vitamin B_7, vitamin H and previously as bios II, factor X or coenzyme R.

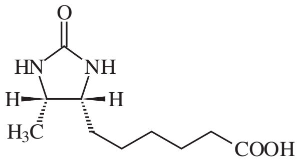


5-84, (+)-biotin

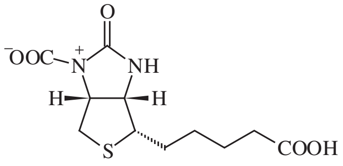
5.11.2 Biochemistry

Most microorganisms, fungi and higher plants synthesise biotin from pimelic acid and alanine. Pimelic acid is a product of oxidative cleavage of higher fatty acids. The primary product of the biosynthesis is dethiobiotin (**5-85**), which reacts with methionine and gives biotin. Biotin is found as a prosthetic group

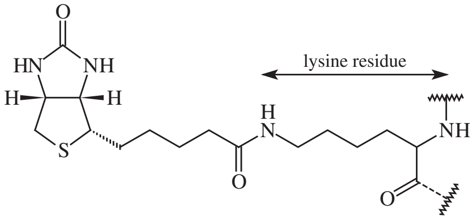
of many enzymes that catalyse the transfer of carbon dioxide. An intermediate of these reactions is *N*-carboxybiotin (**5-86**). Biotin is bound in enzymes through an amide bond provided by the ε-amino group of lysine as the so-called *N*-ε-biotinyl-L-lysine (biocytin, **5-87**). Usually, three groups of enzymes with biotin as a cofactor are recognised: carboxylases, transcarboxylases and decarboxylases. They act in the biosynthesis of fatty acids and the catabolism of branched chain amino acids.



5-85, dethiobiotin

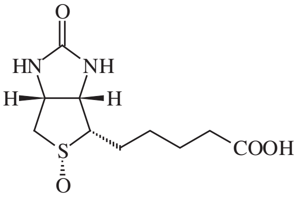


5-86, *N*-carboxybiotin

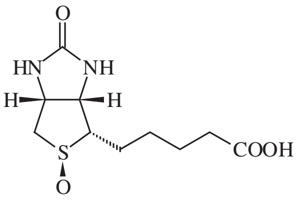


5-87, biocytin

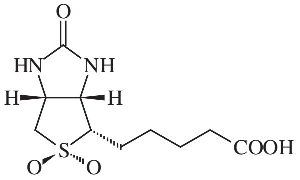
Only free biotin is absorbed from foods. Biotin bound to proteins must be hydrolysed by the enzyme biotinidase (biotinamide amidohydrolase). Excess free biotin is excreted in urine, along with its metabolites (such as the oxidation products biotin sulfoxides, **5-88** and **5-89**). Bound forms of biotin are excreted in the faeces. (+)-Biotin sulfone (**5-90**), also known as *d*-biotin-*d*-sulfone, is the (5*S*)-enantiomer.



5-88, (+)-biotin sulfoxide



5-89, (–)-biotin sulfoxide



5-90, biotin sulfone

5.11.3 Physiology and nutrition

The need for biotin is very low and is usually covered by the vitamin from food and that produced by intestinal microflora. The daily dietary intake is estimated at 50–100 μg, although some sources have reported an even higher intake (150–300 μg). The recommended daily intake for infants (up to 6 months of age) is 5 μg; for infants up to 7–12 months it is 7 μg; for children from 1 year to 3 years, 8 μg; for older children from 4 to 8 years, 12 μg; from 9 to 13 years, 20 μg; for adolescents (14–18 years), 25 μg; for elderly people (also for pregnant women) 30 μg; and for lactating women 35 μg. Spontaneous deficiency is rare and mild. The most common deficiency occurs when the diet is mainly based on the consumption of raw eggs, because raw egg white contains the basic, water-soluble glycoprotein avidin, which forms a very strong complex with biotin in which the vitamin is bound by non-covalent bonds. Biotin bound by avidin (avidin is a biotin antagonist) is not available. Denatured protein does not react with biotin. Vitamin deficiency and avitaminosis manifests by hair loss (alopecia), conjunctivitis (also called red eyes), dermatitis (red rash around the eyes, nose, mouth and genital area) and in adults by neurological symptoms (depression, lethargy and hallucinations).

5.11.4 Use

The enrichment of foods with biotin occurs only rarely.

5.11.5 Occurrence

Biotin is found in a range of foods, but the concentration in most of them is usually low (Table 5.8). It is partly present as a free compound (milk, fruits and vegetables) and partly bound to proteins (animal tissues, plant seeds and yeast). Yeast autolysates, for example, contain free biotin and its precursors and analogues. These biotin vitamers, mainly dethiobiotin (**5-85**), biotin sulfone (**5-90**) and biocytin (**5-87**), are degradation products of enzymes containing biotin.

5.11.5.1 Foods of animal origin

Good sources of biotin are egg yolk and organ meats (especially liver and kidney). Milk has a lower vitamin content.

5.11.5.2 Foods of plant origin

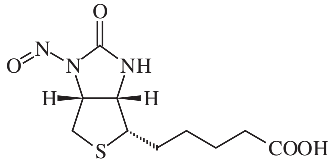
Rich sources are some vegetables, such as peas and cauliflower (about 0.1 mg/kg), cereals, cereal products and legumes. The biotin content in flour is strongly dependent (as is the content of all B group vitamins) on the flour extraction rate. In white flour a little over 10% of the original vitamin present remains in the grain. However, only a small proportion of biotin in wheat is available, better availability comes from maize and soybeans.

5.11.5.3 Other sources

A high level of biotin is found in yeast and mushrooms (about 0.2 mg/kg).

5.11.6 Reactions

Biotin is stable when heated, in the light, in neutral and strongly acidic solutions. It is unstable in alkaline media. It is easily oxidised by hydrogen peroxide and other oxidants to a mixture of isomeric (+)- and (−)-sulfoxides (**5-88** and **5-89**), eventually to biotin sulfone (**5-90**). Sulfoxides also form as metabolic products of microorganisms. They are fully available, whereas sulfone is unavailable as a vitamin. Nitrogen of a ureido ring may be nitrosated to nitrosobiotin (**5-91**) in the presence of nitrites or nitrogen oxides.



5-91, nitrosobiotin

5.11.7 Changes in foods

Biotin is very stable during food processing. Losses in hydrothermal processes are mainly caused by leaching into the water.

5.11.7.1 Meat and meat products

Biotin retention in cooked meat is relatively high, at about 80%.

5.11.7.2 Milk and dairy products

Pasteurisation of milk results in 10–15% losses of biotin. Milk drying losses are higher, but subsequent storage does not influence the vitamin level substantially. Biotin concentration in cheeses is lower by about 20–35% compared with milk. The biotin content of yoghurts is influenced by the microflora (*Lactobacillus* spp.) used, and is lower than in milk by about 45–60%. Some bacteria (e.g. bacteria of the genus *Micrococcus*) produce biotin, and its content in yoghurt may then increase by 5–25%.

5.11.7.3 Cereals and cereal products

Biotin losses during the baking of bread and other cereal products are small. Cooking legumes results in loss of vitamin, which reaches 5-15%, depending on the time of soaking.

5.11.7.4 Fruits and vegetables

During preservation of fruit and vegetables about 30% of biotin is lost. This large proportion is caused by leaching and can therefore be regarded as a loss only when the brew is not consumed.

5.12 Folacin

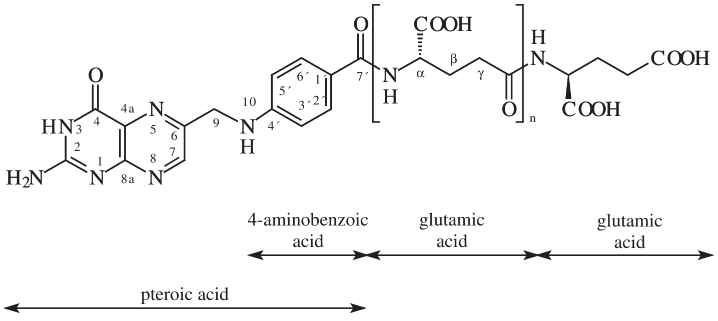
5.12.1 Structure and terminology

Folacin is the name for the biologically active derivatives of folic (pteroylglutamic) acid (**5-92**), formerly also known as vitamin B_9, vitamin B_c or vitamin M. The basis of its structure is pteroic acid, systematic name 4-[(pteridine-6-ylmethyl)amino]benzoic acid, which is hypothetically derived from 6-hydroxymethylpterin and 4-aminobenzoic acid. The carboxyl group of pteroic acid is conjugated, via an amide bond, with one or more L-glutamic acid units, typically with three to eight molecules ($n = 3–8$). These compounds are called glutamyl peptides or folate polyglutamates. The active form is (6*S*)-5,6,7,8-tetrahydrofolic or (6*S*)-5,6,7,8-tetrahydropteroylglutamic acid (**5-93**), for short H_4PteGlu or FH_4, with a reduced pteridine (more precisely pyrazine) ring. Heptaglutamate was previously known under the names of chick growth factor or vitamin B_{11}.

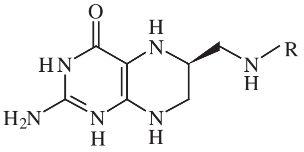
5.12.2 Biochemistry

Many microorganisms and plants are capable of *de novo* synthesis of 7,8-dihydrofolic (7,8-dihydropteroylglutamic) acid (7,8-H_2PteGlu, **5-94**). Animals can only reduce this form of vitamin to tetrahydrofolic acid. They are also able to reduce the folic acid to dihydrofolic acid and can hydrolyse or add additional glutamic acid residues. The starting compound for the biosynthesis of 7,8-dihydrofolic acid is guanosine 5′-triphosphate (GTP). Reactions with 4-aminobenzoic acid (which results from chorismic acid via 4-amino-4-deoxychorismic acid) and with glutamic acid proceed in the later stages of biosynthesis.

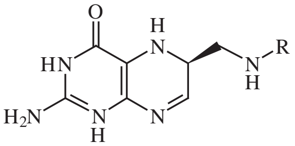
The activity of folacin is similar to the activity of cobalamins. It is linked with the transfer of one of the carbon functional groups, such as methyl (CH_3–), methylene (–CH_2–), formyl (–CH=O) and other groups whose donor is mainly choline, glyoxylic acid, serine and other compounds. These functional groups are bound



5-92, folic acid

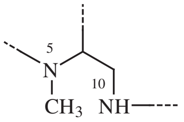


5-93, tetrahydrofolic acid (H_4PteGlu, for R see **5-87**)

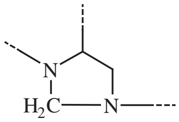


5-94, 7,8-dihydrofolic acid (7,8-H_2PteGlu, for R see **5-87**)

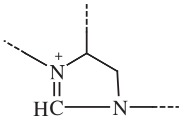
to N-5 or N-10 of tetrahydrofolic acid (**5-95** to **5-100**). Vitamin A is a cofactor for enzymes that act mainly in the metabolism of amino acids (e.g. transamination reactions and synthesis of creatine), purine and pyrimidine nucleotides. Folacin, along with corrinoids, has an important role in homocysteine metabolism, which is one of the risk factors for the origination and development of cardiovascular diseases.



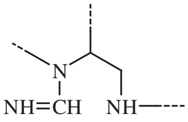
5-95, 5-methyl-H_4PteGlu



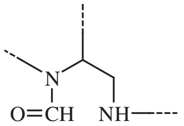
5-96, 5,10-methylen-H_4PteGlu



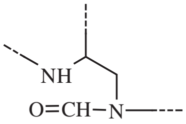
5-97, 5,10-methenyl-H_4PteGlu



5-98, 5-formimidoyl-H_4PteGlu



5-99, 5-formyl-H_4PteGlu



5-100, 10-formyl-H_4PteGlu

5.12.3 Physiology and nutrition

The recommended daily intake of folic acid is 0.065–0.6 mg. Intake at the lower limit is recommended for infants (0.065 mg, 0–6 months; 0.08 mg, 7–12 months) and young children and teenagers (0.15 mg, 1–3 years; 0.2 mg, 4–8 years; 0.3 mg, 9–13 years), the upper income limit is recommended for teenagers, older men and women (0.4 mg; 14–19 years) and pregnant and lactating women (0.6 mg).

Daily intake of 0.6 mg or higher can no longer be obtained from food due to our eating habits, so intake through food supplements is therefore recommended. Because of the difference in bioavailability between folic acid in food supplements and the different forms of folacin found in food, the dietary folate equivalent (DFE) system was established. One DFE is defined as 1 μg of dietary folacin or 0.6 μg of supplemented folic acid. The tolerable Upper intake Level (UL) for folate is 1 mg for adult men and women and 800 μg for pregnant and lactating (breast-feeding) women under 18 years of age. Supplemental folic acid should not exceed the UL to prevent folic acid from masking symptoms of cobalamine deficiency.

Folacin deficiency may lead to glossitis, diarrhoea, depression and confusion. Deficiency anaemia may develop especially in pregnancy and in elderly people. Symptoms of deficiency are similar to symptoms of cobalamine deficiency (known as macrocytic anaemia). Megaloblastic anaemia, the most common cause of macrocytic anaemia, is due to a deficiency of either cobalamine or folic acid (or both). Deficiency in the early stages of pregnancy can lead to developmental defects of the foetus (spinal cord defects and incomplete development of the brain). Women who are at increased risk will need increased daily intake of folic acid.

5.12.4 Use

For enrichment of foods, relatively stable synthetic forms of the vitamin are used, such as 5-formyltetrahydrofolic (**5-99**), 5-methyltetrahydrofolic (**5-95**) and tetrahydrofolic (**5-93**) acids.

5.12.5 Occurrence

Folacin exists primarily in the form of reduced tetrahydrofolates (**5-93**) containing a variable number of glutamic acid units. For example, oranges contain predominantly pentaglutamate (about 40% of the total vitamin content) and tetraglutamate (about 10%), the main form in lettuce is pentaglutamate and monoglutamate (both forms represent about 30% of the vitamin content), the main form in most leafy vegetables is heptaglutamyl conjugate, and fresh meat vitamin consists mainly of penta-, hexa- and heptaglutamate, the major form in stored meat is triglutamate.[9] Fresh foods mostly contain 5-methyltetrahydrofolate (**5-95**) and 10-formyltetrahydrofolate (**5-100**). The total vitamin content in common foods is given in Table 5.8.

5.12.5.1 Foods of animal origin

Particularly important sources of folacin are eggs and offal. The main natural forms of folacin in animal materials are polyglutamylpeptides (the dominating form is the pentaglutamyl conjugate) derived from 5-methyltetrahydrofolic acid, abbreviated to 5-methyl-H_4PteGlu$_n$ (about 50%), which is followed by 10-formyltetrahydrofolic acid, abbreviated to 10-formyl-H_4PteGlu$_n$ (about 10%) and tetrahydrofolic acid or H_4PteGlu$_n$ (about 40%). In milk and dairy products, 25% of the total amount of vitamin is

[9] Plant and animal organisms contain a large number of pteridine derivatives. They act as germination stimulants, for example, in potatoes and soybeans or as pigments in the wings of butterflies (see Section 9.3.4). Animal organisms contain about 100 different forms of folacin.

5-methyl-H_4PteGlu$_n$, about 60% is 10-formyl-H_4PteGlu$_n$ and 15% is H_4PteGlu$_n$.

5.12.5.2 Foods of plant origin

In intact plant tissue, folates are derived from 5-methyl-H_4PteGlu$_n$ and 10-formyl-H_4PteGlu$_n$. These vitamers exist to a small extent as monoglutamates but are mainly conjugated to a chain of 2–8 glutamate moieties. Hydrolysis of folate poly-γ-glutamates to shorter poly-γ-glutamates and monoglutamates is catalysed by endogenous γ-glutamyl hydrolase. For example, 5-methyltetrahydrofolate is the predominant vitamer in sweet peppers (*Capsicum annuum*), but a significant amount of 5-formyltetrahydrolfolate and some 10-formylfolate are also present. Folate content in red and green peppers differs markedly (0.70–0.21 mg/kg, respectively). A significant amount of folic acid (PteGlu$_n$) and its derivatives also occurs in cereals and legumes. In wheat and rye (also in bread and other cereal products and legumes), for example, 38–55% of the vitamin is in the form of 10-formyl-H_4PteGlu$_n$, 5–20% as 5-methyl-H_4PteGlu$_n$ and 3–8% in the form of H_4PteGlu$_n$. In addition, 12–21% of folacin is present as 10-formyl-PteGlu$_n$ and 12–23% as PteGlu$_n$. A rich source of folate are rosehips. Their folate content is 4–6 mg/kg based on dry matter and 1.60–1.9 mg/kg based on the fresh weight (edible part). Fresh citrus juices contain relatively high amounts of mono- and polyglutamyl forms of 5-methyl-H_4PteGlu (0.01–0.13 mg/l) and folic acid (0.07–0.21 mg/l). The concentration of 5-methyl-H_4PteGlu is generally greater than that of folic acid in orange juice, grapefruit juice contains the least amount of 5-methyl-H_4PteGlu but relatively large quantities of folic acid. A relatively high amount (60%) of the 5-methyl-H_4PteGlu is present as the monoglutamyl form. Good folate sources are also strawberries. Their folate content in different strawberry cultivars varied from 0.30 to 0.69 mg/kg fresh weight.

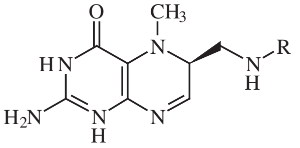
5.12.5.3 Other sources

Rich sources of dietary vitamin are yeast and higher fungi (Basidiomycetes). The dominating folate forms in commercial dry baker's yeast were found to be tetrahydrafolate and 5-methyltetrahydrofolate with a total folate content of 28.9 mg/kg.

5.12.6 Reactions

Folacin is unstable in acid, neutral and alkaline solutions, at higher temperatures and especially in the light in the presence of oxygen, in the presence of transition metals and riboflavin. Stability varies depending on the number of bound glutamate residues and groups at either N-5 or N-10, or a bridge that connects these two positions. Folic and 5-formyltetrahydrofolic acids are relatively stable against oxidation, and less stable is 5-methyltetrahydrofolic acid. Oxidation and other reactions commonly occur during processing and storage of food are shown in Figure 5.23.

In fresh vegetables 5-methyl-H_4PteGlu$_n$ is oxidised in acidic solutions creating unstable but bioavailable vitamin 5-methyl-5,6-dihydrofolic acid (5-methyl-5,6-H_2PteGlu$_n$, **5-101**). This compound isomerises in acidic solutions (during cooking or in the acidic environment of the stomach) to 5-methyl-5,8-dihydrofolic acid (5-methyl-5,8-H_2PteGlu$_n$, **5-102**), which is not bioavailable. Both these forms can be reduced to 5-methyl-H_4PteGlu$_n$ by ascorbic acid. 10-Formyl-H_4PteGlu$_n$ is easily oxidised to 10-formyl-PteGlu$_n$.



5-101, 5-methyl-5,6-dihydrofolic acid (for R see **5-87**)

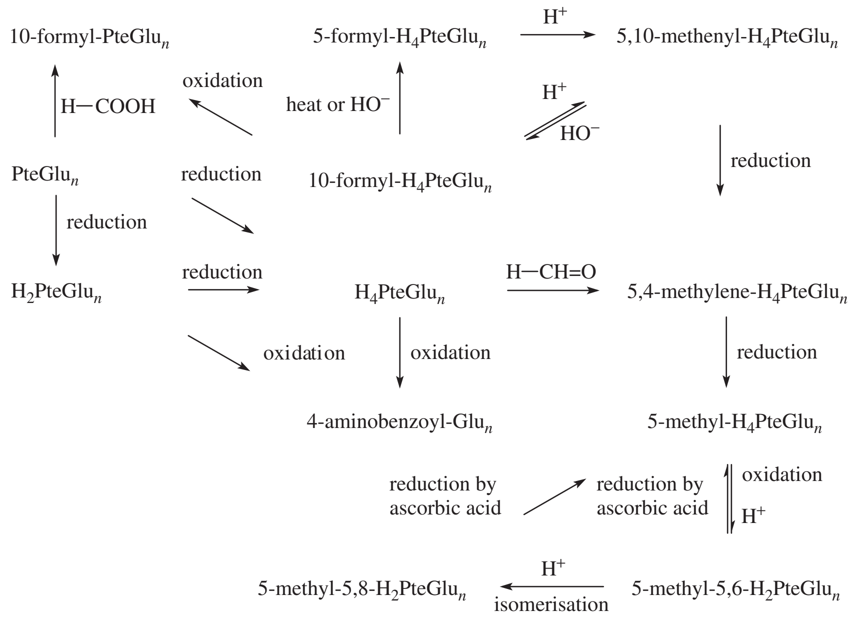
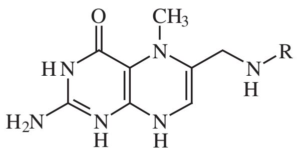


Figure 5.23 Non-enzymatic reactions of folylpolyglutamates.



5-102, 5-methyl-5,8-dihydrofolic acid (for R see **5-87**)

During heating and in an alkaline environment, this compound spontaneously isomerises to 5-formyl-H_4PteGlu$_n$.

Folate degradation produces many different products, depending on pH and other factors. For example, tetrahydrofolic acid (H_4PteGlu) yields 4-aminobenzoyl-L-glutamic acid, 7,8-dihydrofolic acid and a number of simple pterins (Figure 5.24). In the presence of reducing sugars, folic acid may react with their degradation products, such as, the non-enzymatic glycation of folic acid by 1,3-dihydroxyacetone yields the corresponding N^2-[1-(carboxyethyl)]folic acid (Figure 5.25).

5.12.7 Changes in foods

5.12.7.1 Meat and meat products

Meat contains free folacin and folacin bound to polysaccharides. Losses during heat treatment can be as high as 95%. Most of the losses, as in other processed foods, are caused by leaching into water.

5.12.7.2 Milk and dairy products

The stability of folacin in milk depends on the presence of oxygen. Typical losses induced by pasteurisation reach 5%, losses during the production of UHT milk are 10–20%, and condensed milk contains about 75% of the vitamin of the original milk. Folacin content in yoghurt depends on the type of microorganisms used. It may be lower, but also higher than in the original milk. Hard cheeses contain 75–90% of folacin present in the raw material.

5.12.7.3 Cereals and cereal products

Folacin content in cereals is, just like the content of all B group vitamins, highest in the surface layers of the grain. The vitamin content of flour depends on the flour extraction rate. During the preparation of dough, this content does not change, but losses occur during bread baking (20% and more). Cooked pasta products lose about 20% of their folacin content.

5.12.7.4 Fruits and vegetables

When cooking and preserving vegetables, the vitamin loss is about 20–50%.

5.13 Corrinoids

5.13.1 Structure and terminology

Corrinoids are compounds with the activity of vitamin B_{12} that have the most complicated structure of all vitamins. The building block of corrinoids is the corrin ring (**5-103**), a partially hydrogenated, almost planar structure containing four reduced pyrrole rings joined into a macrocyclic ring by links between their α-positions,

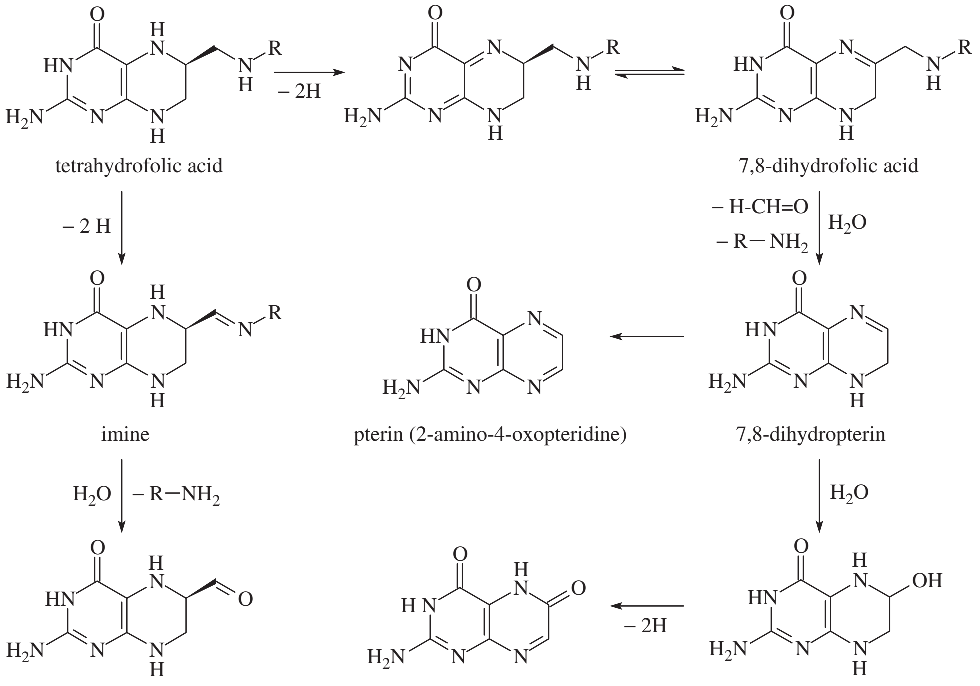


Figure 5.24 Degradation of tetrahydrofolic acid (for R see **5-87**).

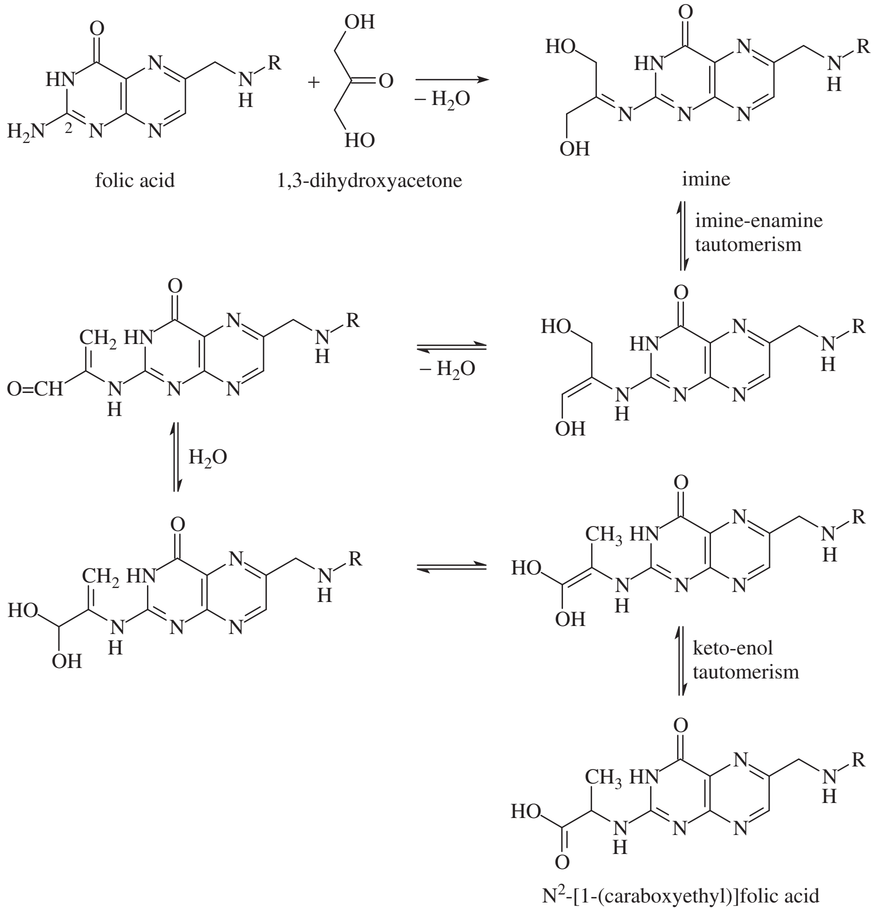
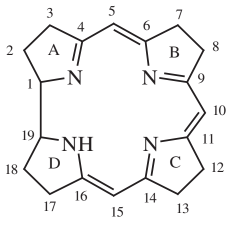


Figure 5.25 Reaction of folic acid with 1,3-dihydroxyacetone. Schneider *et al.* 2002, fig 5. Reproduced by permission of the American Chemical Society.

which resembles the porphyrine ring of haem and chlorophyll pigments. It differs from them, however, as three of these links are formed by one-carbon units (methylidene bridges) as in a porphyrine ring, but the other is by a direct Cα–Cα bond, therefore this structure does not contain the methylidene bridge between pyrrole rings A and D.

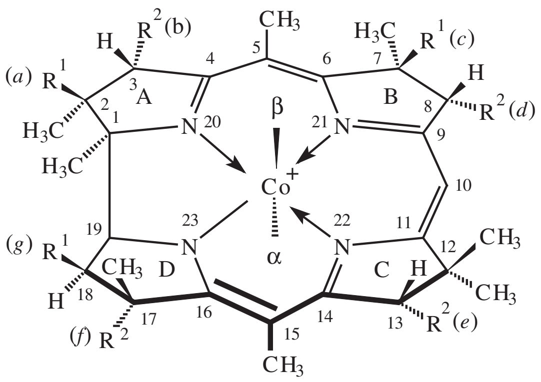


5-103, corrin

Substituents of the corrin ring are mostly methyl groups (six on pyrrole nuclei and two in positions 5 and 15) and amide residues, which include four propionamide residues R^2 (*bdef*) and three acetamide residues R^1 (*acg*) (**5-104**). The orientations of propionamide residues (R^2) are in the so-called α-orientation, while the acetamide residues have β-orientation. Six of the seven carboxamide groups are primary amides, the rest of the propionamide in cycle D, which is R^2 (*f*), has a complicated structure, because it is an amide of propionic acid with (*R*)-1-aminopropane-2-ol that is esterified by α-D-ribofuranosyl-5,6-dimethylbenzimidazole 3′-phosphate.

The central cobalt atom can generate up to six coordination bonds with ligands. It is coordinated to four nitrogen atoms of the pyrrole nuclei; the fifth coordination bond bounds the second nitrogen atom in 5,6-dimethylbenzimidazole in the so-called α-position. In some corrinoids, this position is not occupied. The forms of vitamin B_{12} containing 5,6-dimethylbenzimidazole are called cobalamins. Cobalamins are the only important natural substances that contain a covalent bond C–Co. Cobalt can have an oxidation degree, 3+, 2+, or 1+ according to substitution and biochemical function. For example, cyanocobalamin, hydroxycobalamine, aquacobalamin, methylcobalamin and some other compounds contain Co^{3+} and have a red colour. Enzymatic reduction (also the reaction with thiols) gives a brown product containing Co^{2+} and further reduction yields a grey–green product containing Co^{1+}. Substitution of cobalt by other metals leads to inactive products.

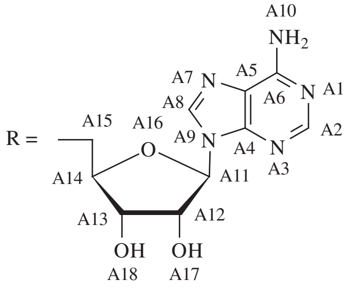
The sixth coordination bond in the β-position can occupy different groups or compounds, or this position may be not occupied at all. The molecule of naturally occurring cobalamine contains

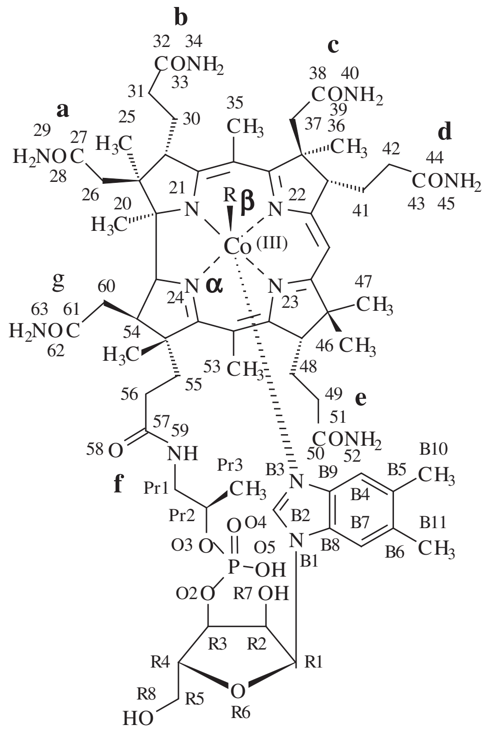


5-104, precursors of cobalamins
cobyrinic acid, R^1=–CH_2–COOH, R^2= CH2–CH_2–COOH
cobyric acid, R^1=–CH_2–CO–NH_2, R^2 (*bde*)=–CH_2–CH_2–CO–NH_2, R^2 (*f*)=–CH_2–CH_2–COOH
cobynic acid, R^1=–CH_2–COOH, R^2=–CH2–CH2–COOH, R^2 (*f*)=–CH_2–CH_2–CO–NH–CH_2–CHOH–CH_3
cobinamide, R^1 =–CH_2–CO–NH_2, R^2 (*bde*)=–CH_2–CH_2–CO–NH_2, R^2(*f*)=–CH_2–CH_2–CO–NH–CH_2–CHOH–CH_3

adenosine, and correspondingly a 5′-deoxy-5′-adenosyl residue, bound through the carbon C-5′. This biologically active form of vitamin B_{12} is called adenosylcobalamine, adenosylvitamin B_{12} or coenzyme B_{12} (for short CoE-B_{12}). Another naturally occurring coenzyme is methylcobalamine that has a methyl group as a ligand (methylvitamin B_{12}). Other ligands are a hydroxyl group (hydroxycobalamine, vitamin B_{12a}), water (aquacobalamine, vitamin B_{12b}) or a nitro group (nitritocobalamine, vitamin B_{12c}). Synthetic vitamin B_{12} known as cyanocobalamine (metabolised to the active substance coenzyme B_{12}) is the form used in pharmaceutical preparations. It contains cyanide anion as a ligand. Cyanocobalamine can occur in the body in cases of cyanide poisoning. Depending on the reaction conditions, other ligands, such as sulfite anion in sulfitocobalamine, can also be bound to the carbon C-5′ as ligands. All these structural analogues based on the corrin skeleton are collectively known as corrinoids (**5-105**).

5´- deoxy-5´-adenosylcobalamine, R = 5´-deoxy-5´-adenosyl
cyanocobalamine, R = CN
hydroxycobalamine, R = OH
aquacobalamine, R = H_2O
methylcobalamine, R = CH_3
nitritocobalamine, R = NO_2
sulfitocobalamine, R = SO_3





5´-deoxy-5´-adenosyl

5´-deoxy-5´-adenosylcobalamine

5-105, structures of important corrinoids

5.13.2 Biochemistry

Vitamin B_{12} is synthesised by many bacteria, some cyanobacteria and even some yeasts. The starting compound is 5-aminolaevulinic (5-amino-4-oxopentanoic) acid. The initial reaction sequences are the same as in the biosynthesis of porphyrins.

Vitamin produced by intestinal bacteria in the gastrointestinal tract of herbivores is absorbed in the tissues and becomes the main vitamin source for other animals in the food chain. Absorption of the vitamin produced by bacteria in the digestive tract of other animals is very small. Cobalamins found in the diet are mostly bound to proteins and released from their protein complexes through the action of acids or pepsin in the stomach. Then, in a unique process, R-protein (also referred to as cobalophilin, haptocorrin and transcobalamine I) secreted in saliva and in gastric juice, picks up the vitamin and transports it through the stomach into the small intestine, where the vitamin is liberated by the action of pancreatic proteases. The free vitamin then attaches to a glycoprotein produced by the parietal cells of the stomach, known as intrinsic factor (abbreviated IF) or gastric intrinsic factor (GIF), that carries the vitamin to the last section of the small intestine, the ileum, the cells of which contain receptors for the cobalamine-IF complex. In supplements, vitamin B_{12} is not bound to protein and therefore does not need digestive enzymes or stomach acid to be detached from protein. In addition to the IF mechanism, passive diffusion normally accounts for 1–3% of vitamin B_{12} absorbed when obtained through food. Another source of vitamin B_{12} can be food contaminated by bacteria producing vitamin B_{12}.

Coenzymes B_{12} as cofactors catalyse two completely different types of reactions. Methylcobalamine (as with folacin) acts in some transmethylation reactions (such as biosynthesis of methionine from homocysteine), collaborates with folic acid in the synthesis of DNA and red blood cells (biosynthesis of porphyrins) and in the fixation of carbon dioxide by some anaerobic acetogennic microorganisms. Enzymes using 5′-deoxy-5′-adenosylcobalamine catalyse a number of isomerisations that are otherwise only viable with difficulty (1,2-rearrangements, such as the formation of succinyl-CoA from methylmalonyl-CoA) and in some organisms they reduce ribonucleotides to deoxyribonucleotides.

5.13.3 Physiology and nutrition

Vitamin B_{12} is not absorbed very well, so relatively large amounts need to be supplied through the diet. The daily intake is estimated at 3–31 μg and about 20–70% is absorbed. The amount of vitamin B_{12} actually needed by the body of an adult is very small, probably only about 2–3 μg/day (the estimated average requirement is 2 μg/day; recommended dietary allowance is 2.4 μg/day), while pregnant and lactating women need 4 μg/day. The need is met mainly by meat, meat products and offal (about 70%; the richest dietary sources are the liver and kidney), followed by milk and dairy products (about 20%), eggs (about 9%) and cereal products (about 2%). Vegetables and fruits are very poor sources as they contain vitamin B_{12} only if contaminated by faecal bacteria.

In contrast to other water-soluble vitamins, vitamin B_{12} is not excreted quickly in the urine, but rather accumulates and is stored in the liver, kidney and other body tissues. As a result, vitamin B_{12} deficiency may not manifest itself until after 5 or 6 years of a diet supplying inadequate amounts. Since plants have no ability to synthesise vitamin B_{12}, strict vegetarians (vegans) have a greater risk of developing vitamin B_{12} deficiency and, hence, need to depend upon vitamin B_{12}-fortified foods or vitamin B_{12}-containing dietary supplements to meet their requirement. Cyanobacteria (blue–green bacteria) of the genus *Spirulina* (*Arthrospira* is in fact correct, nevertheless the older term *Spirulina* remains in use for historical reasons) is a minimal source of vitamin B_{12} and mostly contains the so-called pseudovitamin B_{12}, a derivative that is inactive in humans. Vitamin B_{12} production in higher mushrooms is controversial. It has been suggested that the source of vitamin B_{12} in mushrooms is the microorganisms living on the surface of the mushrooms or the compost containing horse manure–wheat straw that is used for cultivating mushrooms. For example, the highest vitamin B_{12} content in cultivated white button mushrooms (*Agaricus bisporus*) is found in the peel (0.7–3.5 μg/kg) than in the cap, stalk or flesh (0.07–0.8 μg/kg). Lacto-ovo vegetarians usually get enough vitamin B_{12} through consuming milk, dairy products and eggs. Many elderly people (or people after stomach resection or peptic ulcer disease operations) are also deficient because their production of the intrinsic factor needed to absorb the vitamin from the small intestine declines rapidly with age. People with intrinsic factor defects eventually develop a very serious pernicious (deadly) anaemia that manifests by a reduction of haem synthesis.

5.13.4 Use

Vitamin B_{12} is used for the enrichment of some foods (such as breakfast cereals, soy products, energy bars and yeast extract spread) and which may be the source of corrinoids for strict vegetarians and vegans. Along with other vitamins, vitamin B_{12} is added to many multivitamin preparations and to food supplements. Cyanocobalamin, which is used in most supplements, is readily converted into the coenzyme forms of cobalamin (methylcobalamin and 5′-deoxyadenosylcobalamin) in the human body.

5.13.5 Occurrence

5.13.5.1 Foods of animal origin

Corrinoids are present almost exclusively in foods of animal origin (Table 5.9). In milk, the main vitamins are adenosylcobalamine and methylcobalamine; cheeses and egg yolk contain mainly methylcobalamine.

5.13.5.2 Foods of plant origin

Cobalamine does not occur in plants, although its presence has been acknowledged in legumes. Occasional findings in vegetables or fermented foods (such as beer, miso and soy sauce) may originate from contamination by organic fertilisers or by wild types of

Table 5.9 Corrinoids content of selected foods.

Food	Edible portion (μg/kg or μg/l)	Food	Edible portion (μg/kg or μg/l)
Pork meat	6–10	Fish	13–28
Beef meat	112–20	Clams	11
Chicken meat	2–5	Milk	3–38
Pork liver	260–1220	Cheese	6–33
Beef liver	590–830	Eggs	7–9
Pork kidney	85–200	Yeast extract spread	5

microorganisms. The vitamin probably comes from the biomass of these microorganisms. It is synthesised by many bacteria and some yeasts, such as *Candida utilis*.

5.13.6 Reactions

Despite their complex structure, cobalamins are relatively stable in solution at pH 4–7. In acidic solutions, cyanocobalamine gives hydroxycobalamine, which in acidic and neutral solutions exists as aquacobalamine. Acid hydrolysis of corrinoids produces mono-, di- and tricarboxylic acids from the bound propionamides. Most susceptible to hydrolysis is the propionamide in position *e*. Hydrolysis of acetamide chains requires more drastic conditions, which simultaneously release the nucleotide and isopropylamine group in position *f*. Altogether, the acidic hydrolysis releases six molecules of ammonia. Hydrolysis in dilute solutions of alkali metal hydroxides yields biologically inactive dehydrovitamin B_{12} (the biologically inactive form with a lactam ring attached to ring B of the cobinamide ring). Alkaline hydrolysis further produces cyanocobinamide, 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole and phosphate. Under alkaline conditions a *c*-lactone ring in ring B also results.

Photodegradation of adenosylcobalamine in the absence of oxygen gives cobalamine and 5,8-cycloadenosine. In the presence of oxygen, aquacobalamine and adenosine-5′-carbaldehyde are produced. Photolysis of methylcobalamine in aqueous solutions and in the absence of oxygen produces, aquacobalamine and methane as major products, or aquacobalamine and formaldehyde if in the presence of oxygen. In aqueous solutions in the presence of ascorbic acid, aquacobalamine is reduced, hydroxylated at C-5, forms a lactone ring between the carboxyl *c* and C-6 of the corrin ring and yellow xanthocorrinoids are produced as the final products. The hydroxyl group in hydroxycobalamine can be easily substituted by another ligand, such as a cyanide anion and chlorine anion. The corrin ring can also be halogenated at C-10, for example, by chloramine-T (used as a disinfection and sanitation agent). The reaction of methylcobalamine with metal ions in aqueous solutions produces methyl derivatives of metals (transfer of methyl anion CH_3^-) and aquacobalamine.

5.13.7 Changes in foods

During food processing and culinary operations, vitamin B_{12} seems to be very stable. The main reason for the loss is leaching into water.

5.13.7.1 Meat and meat products

Vitamin losses during meat processing are dependent on the technology used. They can reach 55–70% of the original amount of the vitamin.

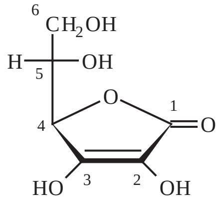
5.13.7.2 Milk and dairy products

Under normal processing conditions, the vitamin content in milk does not change too much. The loss caused by pasteurisation is up to 10% and about 10–20% in UHT milk. In the manufacture of hard cheeses, the vitamin retention is about 60–90% of the original content. Dairy products fermented using *Propionibacterium shermanii* (such as Swiss-type cheeses), have increased vitamin content in comparison with the original milk (up to 30 times).

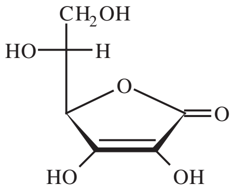
5.14 Vitamin C

5.14.1 Structure and terminology

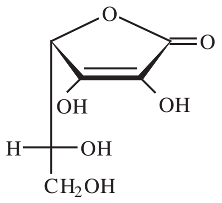
The basic biologically active compound is ascorbic acid. Of the four possible stereoisomers (asymmetric carbons C-4 and C-5), the activity of vitamin C is seen only in L-ascorbic acid (L-*threo*-hex-2-enonic acid 1,4-lactone; formerly called ceritaminic acid, 2-keto-L-gulonic acid, L-*xylo*-hex-2-ulosonic acid and later L-*xylo*-ascorbic acid). The D-isomer of ascorbic acid (D-*xylo*-ascorbic acid) and the second pair of enantiomers, known as L- and D-isoascorbic acids (**5-106** to **5-109**), do not show vitamin C activity. Previously, these acids, which are γ-lactones of L- and D-*erythro*-hex-2-enonic acids, were known as L- and D-erythorbic acids or L- and D-*arabino*-ascorbic acids.



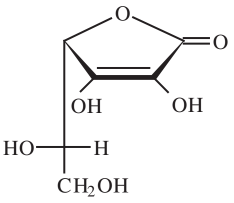
5-106, L-ascorbic acid



5-107, D-isoascorbic acid



5-108, D-ascorbic acid



5-109, L-isoascorbic acid

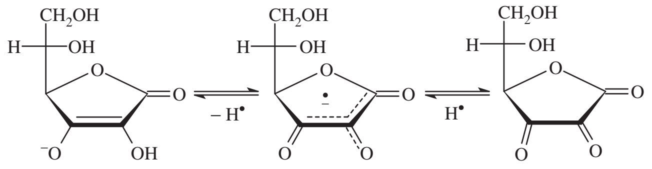


Figure 5.26 Biologically active forms of vitamin C.

The name vitamin C refers not only to L-ascorbic acid, but also to the whole reversible redox system that includes the one-electron oxidation product of L-ascorbic acid, known as L-ascorbyl radical (or L-monodehydroascorbic acid or semidehydroascorbic acid), and the two-electron oxidation product of L-ascorbic acid known as L-dehydroascorbic acid (Figure 5.26). Ascorbic acid and ascorbyl radical mainly occur as anions in solutions at physiological pH.

5.14.2 Biochemistry

Ascorbic acid is synthesised by all green plants that provide their energy needs through photosynthesis (photoautotrophic plants). Almost all living organisms, insects and invertebrates, most fish and many species of birds and mammals, have the ability to synthesise ascorbic acid. Notably, humans, but also gorillas, chimps, orangutans and some monkeys, frutivorous (fruit eating) bats, guinea pigs and capybaras lack this ability.

Plants synthesise ascorbic acid from the active form of D-mannose, which is GDP-D-mannose. In mammals, the starting compound is UDP-D-glucose. Microorganisms and higher fungi synthesise 6-deoxy-L-ascorbic acid from L-fucose and D-erythroascorbic acid from D-arabinose.

The functions of ascorbic acid are related primarily to its redox properties. In plants it has a role in photosynthesis; it regulates the amount of active oxygen species and also acts in the growth and differentiation of cells. Cleavage of the molecule of ascorbic acid also creates specific metabolites, such as L-threonic, L-tartaric, L-glyceric and oxalic acids.

In animals, ascorbic acid is involved primarily in hydroxylation reactions ongoing in the body. The most important oxidation reaction is hydroxylation of proline to 3-hydroxyproline and of lysine to 5-hydroxylysine in procollagen, which is related to protein biosynthesis of connective tissue. Another important reaction is hydroxylation of 3,4-dihydrophenylethylamine (dopamine) to norepinephrine (noradrenaline) and biosynthesis of betaine carnitine. Norepinephrine, along with other so-called catecholamines, acts as a carrier of chemical information (hormone and neurotransmitter); carnitine acts as a carrier of fatty acid residues through the membrane of the mitochondria. Vitamin C also participates in the biosynthesis of mucopolysaccharides, prostaglandins, homogentisic acid (the precursor of tocopherols and plastoquinones), participates in the absorption of ionic forms of iron, its transport, stimulating the transport of sodium, chloride and possibly also of calcium ions, allows the transfer of sulfate in the form of ascorbyl-2-sulfate and acts in the metabolism of cholesterol, drugs and in many other reactions.

Very important reactions related to the antioxidant properties of vitamin C are reactions with active oxygen forms, with free radicals and with oxidised forms of vitamin E, respectively, which protects vitamin E and lipid membranes from oxidation. Vitamin C has a protective function for labile forms of folic acid. It also inhibits formation of nitrosamines and acts in this way as a modulator of mutagenesis and carcinogenesis. Many other activities of vitamin C are still only partially understood or not even at all.

5.14.3 Physiology and nutrition

A daily dose of 10 mg of L-ascorbic acid is sufficient to prevent scurvy. In the past, the recommended daily intake was 30 mg (50 mg for adolescents and 60 mg for pregnant women). Today, recommendations for vitamin C intake have been set by various national agencies and range from 40 to 95 mg/day (e.g. 40 mg are recommended in Great Britain, 45 mg are recommended by the WHO, and 60–95 mg are recommended in the United States). The US-recommended daily dietary intake of vitamin C is 30 mg for infants, 45 mg for children, 60 mg for adult men and women, 80 mg for pregnant women (second and third trimesters) and 100 mg during lactation. For patients with respiratory diseases and during convalescence and in other cases (such as radiation therapy), the daily doses may reach 500–1000 mg or even more. The tolerable upper intake levels for adult men and women are 2000 mg/day. The possible adverse effects of high doses of vitamin C must also be considered. These include the so-called rebound scurvy (the vitamin C dependency state that occurs in the foetus of a woman taking megadoses of vitamin C during pregnancy), increased excretion of oxalic acid in the urine (which may lead to stones in the urinary tract), an increased absorption of iron in those susceptible to iron overload, increased absorption of toxic metals and interference with certain medications (e.g. warfarin, aspirin, antidepressants and contraceptive pills).

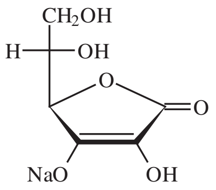
The total vitamin C requirement is met by foods, especially potatoes (that cover about 20–30%), vegetables (about 30–40%) and fruits (30–35%). Milk covers less than 10% of the vitamin need. Deficiency or hypovitaminosis manifests in a number of non-specific symptoms, most commonly known as spring fatigue. The best-known syndrome of acute avitaminosis is scurvy. According to some new findings, dehydroascorbic acid has somewhat lower activity than ascorbic acid.

Oxidoreductases (such as ascorbate oxidase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, superoxide dismutase and ascorbate: cytochrome-*b* reductase) that act in the metabolism of vitamin C in animals and plants can be considered to be antivitamins C. Other oxidoreductases, such as enzymes known trivially as polyphenoloxidases and some others, may indirectly cause loss of ascorbic acid.

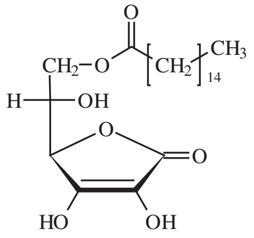
5.14.4 Use

Ascorbic acid is widely used as a food additive due to its properties (vitamin, antioxidant and chelating agent), especially in canning

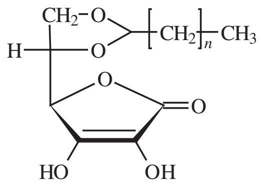
and fermentation processes, but also in meat, fat and cereal technologies. The water-soluble salt of L-ascorbic acid sodium L-ascorbate (**5-110**) and the lipophilic compound 6-palmitoyl-L-ascorbic acid (L-ascorbyl 6-palmitate, **5-111**), which inhibits the formation of nitrosamines in cured meat and meat products, are also used as antioxidants. Non-polar acetals of L-ascorbic acid derived from fatty aldehydes (dodecanal to octadecanal, **5-112**) were used as inhibitors of nitrosamine formation in the production of ham, as they are more stable than ascorbyl palmitate. D-Isoascorbic acid can in many cases (such as an antioxidant) replace L-ascorbic acid, but it cannot be used to improve the baking properties of flour (D- and L-isoascorbic acids are less active than L-ascorbic acid, D-ascorbic acid is completely inactive) because it is a stereospecific reaction.



5-110, sodium L-ascorbate

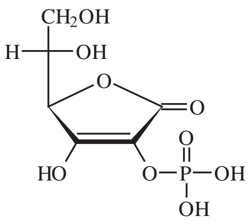


5-111, L-ascorbyl 6-palmitate

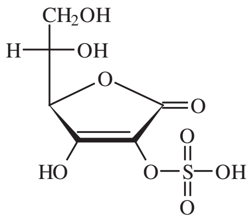


5-112, L-ascorbic acid acetals (n=10–16)

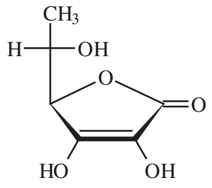
Sodium ascorbate and ascorbic acid esters, such as ascorbyl 6-palmitate (**5-111**) and ascorbyl 2-phosphate (**5-113**), are fully bioavailable, while ascorbyl 2-sulfate (**5-114**) is a completely inactive vitamin form. Phosphate and sulfate are about 20 times more stable to oxidation than the free acid. D-Isoascorbic acid (**5-107**) shows only 5–20% activity, 6-deoxy-L-ascorbic acid (**5-115**), found in fungi, has about 30% activity and the bound ascorbic acid form ascorbigen has 15–20% of the activity of ascorbic acid. Ascorbic acid 2-*O*-β-D-glucoside, systematic name 2-*O*-(β-D-glucopyranosyl)-L-ascorbic acid (**5-116**), has the same biological activity as ascorbic acid and is also stable against oxidation.



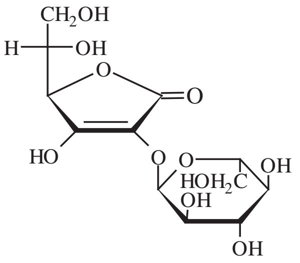
5-113, L-ascorbyl 2-phosphate



5-114, L-ascorbyl 2-sulfate



5-115, 6-deoxy-L-ascorbic acid



5-116, 2-*O*-(β-D-glucopyranosyl)-L-ascorbic acid

5.14.4.1 Fruits and vegetables

Ascorbic acid is added to fruit juices, stewed fruit and frozen fruit to prevent undesirable changes in flavour caused by oxidation during storage and processing. The removal of oxygen in airtight containers requires the addition of 3–7 mg of ascorbic acid per cm^3 of air present (depending on pH and temperature). Ascorbic acid at relatively low concentrations is often used as an inhibitor of the enzymatic browning reactions during peeling, slicing and drying of fruits, vegetables and potatoes. Ascorbic acid is often used in combination with citric acid as it is more stable in acid solutions, and the optimum pH of enzymes (phenolases) lies in the pH range of 6–7. A common practice is soaking the fruits or vegetables for 3 min in a solution containing 1–3% ascorbic acid, 0.1–0.3% calcium chloride and 0.015% hydrogen sulfites (they act as preservatives). In the absence of ascorbic acid, the amount of hydrogen sulfites in the bath has to be increased approximately tenfold.

5.14.4.2 Beer and wine

The addition of 20–30 mg/l ascorbic acid prevents the formation of colloidal turbidity (called chill haze) in beer, and also prevents adverse changes in flavour due to the oxidation that occurs during pasteurisation and storage. The use of ascorbic acid in winemaking can reduce the amount of sulfur dioxide used for fumigation.

5.14.4.3 Meat and meat products

The addition of ascorbic acid (or sodium ascorbate or ascorbyl palmitate) to meat and meat products together with nitrites (at a level of 60–180 mg/kg), for example, in the production of ham, is of functional and economic importance as it enhances and speeds up its production considerably. The characteristic pigment of raw meat treated with nitrites, nitroxymyoglobin, is formed approximately three times faster. The addition of ascorbic acid allows the time of smoking to be shortened and stabilises the colour of the finished

product. Ascorbic acid also enhances the inhibitory effects of nitrite on toxinogenic *Clostridium botulinum* bacteria. Additions of 300–1000 mg/kg of ascorbic acid (the optimal ratio of ascorbate and nitrite is 2 : 1; hydrophilic ascorbate is only partially effective and is often replaced by ascorbyl palmitate, which is soluble in fats) also inhibit the formation of nitrosamines. If nitrates used instead of nitrites, these are reduced to nitrites by ascorbic acid.

5.14.4.4 Bread

The baking properties of flour improve after a certain period of storage, when the products of lipid oxidation react with thiol groups of flour proteins that are oxidised and form disulfide bridges between the protein molecules. Simultaneous degradation of carotenoid pigments results in a lighter colour of the flour. The addition of ascorbic acid (10–100 mg/kg) improves the baking properties of flour, especially during the Chorleywood industrial bread making process, which reduces the time and labour input needed to make bread, thereby reducing manufacturing costs (see Section 5.14.6.1).

5.14.4.5 Fats and oils

Ascorbic acid does not occur in fats and oils, but is used, as ascorbyl palmitate, as an antioxidant in amounts ranging from 0.006 to 0.040%.

5.14.5 Occurrence

Plasma and tissues of animals, as well as of foods of plant origin, usually contain 90–95% of vitamin C as ascorbic acid, the remainder is dehydroascorbic acid.

5.14.5.1 Foods of animal origin

Vitamin C is found in significant amounts only in the liver (Table 5.10). Other foods of animal origin (meat, eggs and milk) have almost negligible importance as sources of vitamin C.

5.14.5.2 Foods of plant origin

More than 90% of vitamin C in the human diet is supplied by fruits and vegetables (including potatoes). Fresh fruits and vegetables are the richest sources of vitamin C, but its amount varies considerably between species and cultivars. The vitamin content in horticultural crops is also strongly dependent on a number of climatic environments, preharvest and postharvest factors, such as preharvest climatic conditions, maturity, harvesting methods and postharvest handling procedures. The absolute highest concentration of ascorbic acid, reaching 17–46 g/kg edible portion, accumulates in the fruit of acerola (*Malpighia emarginata, syn. A. glabra*, Malpighiaceae), also known as Barbados cherry or West Indian cherry. A comparable content of vitamin C, 23–32 g/kg edible portion is in the Australian fruit of *Terminalia ferdinandiana* (Combretaceae), commonly known as Kakadu plum or billygoat plum. Some other excellent sources of vitamin C (such as rosehips, blackcurrant and curly parsley) are usually not too important in meeting the needs of vitamin C, because they are consumed only occasionally and in small quantities. Of much greater importance are the sources with an average or lower level of the vitamin, such as potatoes that are known to be the most important source of vitamin C in the Western diet, because of the large quantities consumed (although the vitamin content in potatoes significantly decreases during storage for the winter months). In winter and spring months, subtropical fruits (especially oranges) fulfil a

Table 5.10 Vitamin C content of selected foods.

Food	Edible portion (mg/kg or mg/l)	Food	Edible portion (mg/kg or mg/l)
Meat	10–20	Carrot	50–100
Ham (pork, cured)[a]	300–500	Parsley (root)	230
Offal	50–340	Parsley (curly)	1500–2700
Milk	5–20	Chive	430
Apples	15–50	Leek	150–300
Pears	20–40	Onion	90–100
Plums	25–45	Garlic	150–160
Peaches	70–100	Horseradish	450–1200
Cherries, sweet cherries	60–300	Cabbage	170–700
Gooseberries	330–480	Cabbage (Savoy)	700–1400
Currant (red)	200–500	Brussel sprouts	1000–1030
Currant (black)	1100–3000	Broccoli	1100–1130
Grapes	20–50	Cauliflower	47–1610
Strawberries	400–700	Kohlrabi	280–700
Blackberries	90	Lettuce	60–300
Melons	130–590	Spinach	350–840
Oranges	300–600	Tomatoes	80–380
Lemons	300–640	Eggplant	80
Grapefruits	240–700	Pepper (various types)	620–3000
Pineapple	150–250	Cucumber	65–110
Bananas	90–320	Asparagus	150–400
Kiwi	700–1630	Peas	80–410
Mango	100–350	Beans	90–300
Papaya	620–980	Beetroot (red)	65
Rosehips	2500–10000	Potatoes	80–400

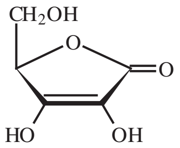
[a] Added ascorbic acid.

considerable portion of the vitamin C requirements. In the past, good sources of vitamin C were common vegetables that could be eaten year round, such as cabbage, but at present many other vegetable are regularly consumed (such as red and green peppers and tomatoes). Cereals contain only traces of vitamin C, somewhat higher levels are found only in germinating seeds.

A precursor of ascorbic acid, 2-*O*-(β-D-glucopyranosyl)ascorbic acid, was recently isolated from both the ripe fresh fruit and dried fruit of *Lycium barbarum* (Solanaceae) known as Chinese wolfberry or Chinese boxthorn, which is native to south eastern Europe and Asia. The dried fruit contains 2-*O*-(β-D-glucopyranosyl)ascorbic acid at a level of 5000 mg/kg, which is comparable to the ascorbic acid content of fresh lemons.

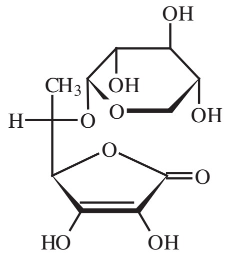
5.14.5.3 Other sources

Fungi, including the so-called higher fungi (commonly referred to as mushrooms), do not contain ascorbic acid; however, they contain a number of structurally related compounds. The main compounds are 6-desoxy-L-ascorbic acid (**5-116**), the five-carbon ascorbic acid analogue D-erythroascorbic acid, which is (4*R*)-2,3,4,5-tetrahydroxypent-2-enonic acid 1,4-lactone, also known as D-*glycero*-pent-2-enonic acid 1,4-lactone or D-*erythro*-pent-2-ulosonic acid 1,4-lactone (**5-117**). These acids occur as free compounds and as C-5 glycosides. 6-Deoxy-L-ascorbic acid, D-erythroascorbic acid, 6-deoxy-5-*O*-(α-D-xylopyranosyl)-L-ascorbic acid (**5-118**), 6-deoxy-5-*O*-(α-D-glucopyranosyl)-L-ascorbic acid (**5-119**), 5-*O*-(α-D-xylopyranosyl)-D-erythroascorbic acid (**5-120**) and 5-*O*-(α-D-glucopyranosyl)-D-erythroascorbic acid (**5-121**) were isolated from some edible fungi (of the phylum Basidiomycota), 5-*O*-(α-D-galactopyranosyl)-D-erythroascorbic acid (**5-122**) occurs in the pathogenic fungus *Sclerotinia sclerotiorum* (of the phylum Ascomycota) and 5-*O*-(α-D-glucopyranosyl)-D-erythroascorbic acid (**5-121**) were isolated from a filamentous fungus *Phycomyces blakesleeanus* (of the phylum Zygomycota). Yeast (*Saccharomyces cerevisiae*) and red bread mould (*Neurospora crassa*, Ascomycota) contain only D-erythroascorbic acid.[10] 6-Deoxyascorbic acid and its glycosides have lower activity than ascorbic acid and D-erythroascorbic acid does not show any vitamin C activity. All these compounds have similar functions in fungi to ascorbic acid in plants and animals, as they act as antioxidants.

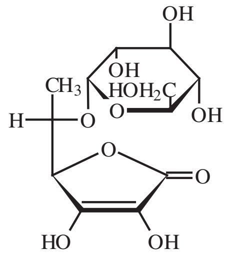


5-117, D-erythroascorbic acid

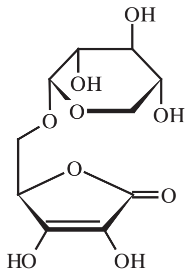
[10]The addition of non-physiological substrates (such as L-galactose) can in some microorganisms (also in the yeast *Saccharomyces cerevisiae*) induce the biosynthesis of L-ascorbic acid by enzymes that are otherwise employed in the biosynthesis of D-erythroascorbic acid.



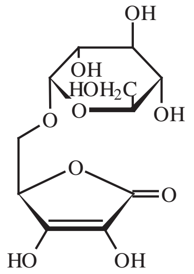
5-118, 6-deoxy-5-*O*-(α-D-xylopyranosyl)-L-ascorbic acid



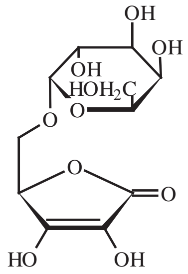
5-119, 6-deoxy-5-*O*-(α-D-glucopyranosyl)-L-ascorbic acid



5-120, 5-*O*-α-D-xylopyranosyl-D-erythroascorbic acid



5-121, 5-*O*-(α-D-glucopyranosyl)-D-erythroascorbic acid



5-122, 5-*O*-(α-D-galactopyranosyl)-D-erythroascorbic acid

5.14.6 Reactions

Both enolic hydroxyl groups of ascorbic acid can dissociate, and ascorbic acid can be considered as a diprotic acid (pK_1 = 4.25; pK_2 = 11.8). In solutions of physiological pH values, the prevailing ion is a monoanion. For example, in solutions of pH 7.4, 99.93% of ascorbic acid is present in the form of a monoanion, the rest are undissociated acid (0.06%) and dianions (0.01%). Only salts of the monovalent anion, such as sodium ascorbate, are known.

Ascorbyl radical is acid (pK = −0.96), which exists in solution as a resonance stabilised anion (as a bicyclic compound, apparently with a double bond between carbons C-2 and C-3) and with the unpaired electron located in the C-4 region. Dehydroascorbic acid occurs as the bicyclic hydrated monomer (3,6-anhydro-L-xylo-hexulono-l,4-lactone hydrate, Figure 5.27) in aqueous solutions.

5.14.6.1 Ascorbic acid

Oxidation of ascorbic acid to dehydroascorbic acid is catalysed by many enzymes (oxidoreductases), which belong to the category of ascorbic acid antivitamins. Ascorbic acid is also oxidised by atmospheric oxygen, hydrogen peroxide and various other oxidising agents. Oxidation to dehydroascorbic acid is a reversible reaction and can be carried out by various mechanisms. The loss of one electron yields a radical of ascorbic acid as an intermediate and the reaction is known as one-electron oxidation. Oxidation of ascorbic acid by the loss of two electrons yields dehydroascorbic acid, which is the first chemically stable product.

5.14.6.1.1 Enzymatic oxidation

Formation of dehydroascorbic acid

The oxidation of ascorbic acid in the enzymatically active and, especially, in the mechanically damaged plant tissues (e.g. by peeling and slicing) is mainly catalysed by ascorbate oxidase (L-ascorbate:O_2 oxidoreductase). The loss of vitamin activity in some plant tissues is associated with peroxidases and other enzymes. Ascorbate oxidase oxidises ascorbic acid in the presence of atmospheric oxygen. Generally, the reaction can be described by the following equation, where H_2A is ascorbic acid and A is dehydroascorbic acid:

$$2\,H_2A + O_2 \rightarrow 2\,A + 2\,H_2O$$

Ascorbate peroxidase employs hydrogen peroxide as a proton acceptor:

$$H_2A + H_2O_2 \rightarrow A + 2\,H_2O$$

Detailed reaction mechanisms are in fact more complex. In both cases, the primary product of oxidation of ascorbic acid is an ascorbyl radical ($HA^{\bullet}$) and its anion ($HA^{\bullet-}$) stabilised by resonance, respectively. It is relatively inert and does not react with oxygen, but comparatively quickly (the reaction half-life is about 0.2 s) provides an equimolar mixture of ascorbic acid and dehydroascorbic acid (or bicyclic dehydroascorbic acid hydrate, Figure 5.27) by disproportionation:

$$2\,H_2A \rightarrow 2\,HA^{\bullet} + 2\,H^{+} + 2\,e^{-}$$
$$2\,HA^{-} + 2\,e^{-} \rightarrow 2\,HA^{\bullet-}$$
$$2\,HA^{\bullet-} \rightarrow H_2A + A + 2\,e^{-}$$

The reaction is repeated until all the ascorbic acid is oxidised. The reaction is reversible and dehydroascorbic acid can be reduced back to ascorbic acid by reduced glutathione, cysteine and other thiols, hydroquinones and other compounds. Dehydroascorbic acid is already fairly unstable and spontaneously hydrolyses with lactone ring opening. This reaction is not reversible. Losses of vitamin caused by enzymatic oxidation in fruits and vegetables during processing can be effectively reduced by blanching using steam or boiling water, which inactivates the enzymes oxidising ascorbic acid.

Formation of oxalic, threonic and tartaric acids

The enzyme ascorbate 2,3-dioxygenase (L-ascorbate:O_2 2,3-oxidoreductase) splits the molecule of ascorbic acid between carbons C-2 and C-3 (this requires triplet oxygen, water and Fe^{2+} ions) to oxalic acid and L-threonic acid, also known as (2*R*,3*S*)-trihydroxybutanoic acid. The oxidative cleavage of ascorbic acid plays an important role in the metabolism in most plants, because, in addition to oxalic acid that plays a role in calcium metabolism, the final product is (+)-L-tartaric acid (Figure 5.28), which is formed by oxidation of threonic acid. Threonic and oxalic acids are the main decomposition products of ascorbic acid also in weakly alkaline solutions and during oxidation by singlet oxygen (Figure 5.29). D-Erythroascorbic acid in fungi decomposes in an anlogous way, yielding oxalic acid and D-glyceric acid.

5.14.6.1.2 Autoxidation and oxidation by singlet oxygen

The most important reaction is the oxidation of ascorbic acid by air oxygen (autoxidation), which mainly causes losses in foods during processing. It takes place in both the presence and absence

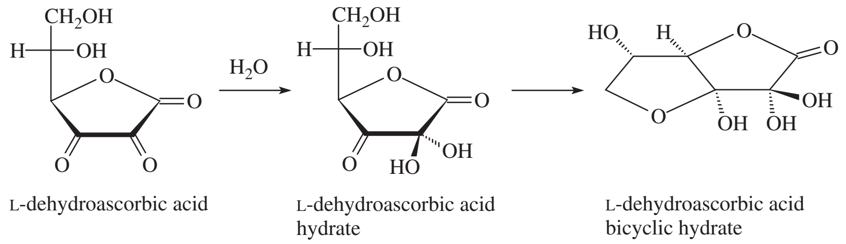


Figure 5.27 Formation of L-dehydroascorbic acid hydrate.

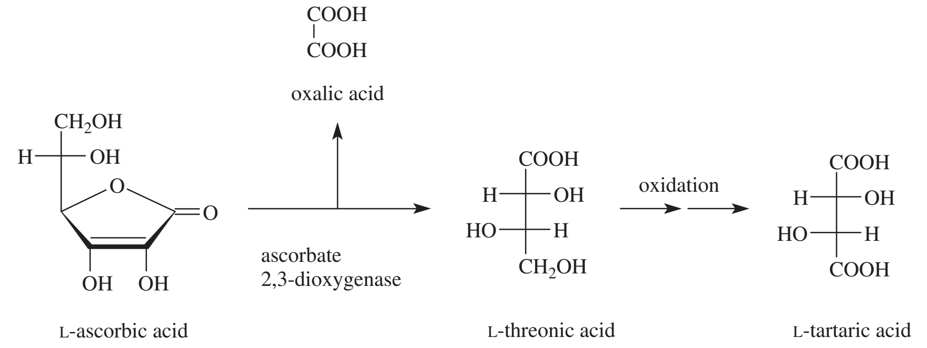


Figure 5.28 Formation of oxalic, threonic and tartaric acids.

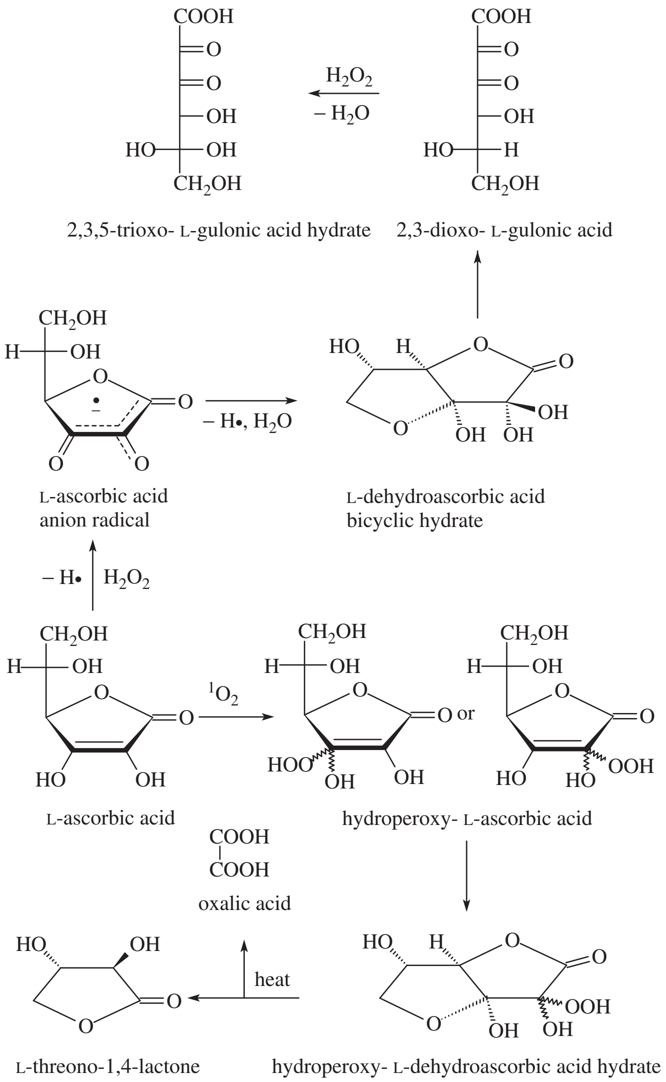
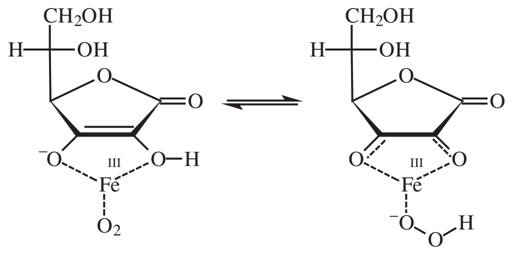


Figure 5.29 Oxidation of ascorbic acid by hydrogen peroxide and singlet oxygen.

of transition metal ions. The most active ions are trivalent iron and divalent copper. The reaction depends on the pH; in an acidic solution it is slow, in a neutral solution it is faster and in an alkaline solution it is fastest. The catalytic efficiency of metal ions lies in the fact that ascorbic acid forms a very stable ternary complex (**5-123**) with a metal ion of higher valency and oxygen (in solutions of pH 2–8) in which it is present as an anion HA^-. Therefore, the reaction rate increases with pH. Other enediols react analogously.



5-123, complex of L-ascorbic acid with ferric ion sand oxygen

Within the complex, two electrons are transferred from ascorbic acid to oxygen through the metal ion. The complex dissociates by the action of hydrogen ions (H^+) forming dehydroascorbic acid, hydrogen peroxide and the metal ion. Reaction with Fe^{3+} (Fe^{2+} complex is less stable than the complex with Fe^{3+}) can be generally described by the following equations (similar reaction takes place with Cu^{2+}):

$$H_2A + O_2 \xrightarrow{Fe^{3+}} A + H_2O_2$$

The resulting hydrogen peroxide can oxidise another molecule of ascorbic acid to dehydroascorbic acid or other oxylabile compounds in foods (such as anthocyanin pigments), but can also react with Fe^{2+} ions to form hydroxyl ions and hydroxyl radicals:

$$H_2A + H_2O_2 \rightarrow A + 2\ H_2O$$

The resulting reaction, which is the sum of the previous two reactions, can be described as follows.

Oxidation of ascorbic acid by hydrogen peroxide proceeds via ascorbyl radical and dehydroascorbic acid (apparently its hydrate), which yields 2,3-dioxo-L-gulonic acid. Hydrogen peroxide further oxidises 2,3-dioxo-L-gulonic acid to give unstable 2,3,5-trioxo-L-gulonic acid (Figure 5.29), which decomposes and produces other

products. 2,3-Dioxo-L-gulonic acid and 2,3,5-trioxo-L-gulonic acid are probably hydrated in solutions.

Oxidation of ascorbic acid by singlet oxygen gives L-threonic acid 1,4-lactone and oxalic acid, analogously to the reaction catalysed by ascorbate 2,3-dioxygenase. Intermediates of this reaction are hydroperoxides of ascorbic acid and of dehydroascorbic acid hydrate (Figure 5.29).

5.14.6.1.3 Reduction of metal ions

Ascorbic acid reacts with metal ions to form complexes, but under certain conditions (especially at low pH values and if present in low concentrations) may also reduce metal ions. Reaction with Fe^{3+} ions (and by analogy with Cu^{2+} ions) is as follows:

$$H_2A + 2\,Fe^{3+} \rightarrow A + 2\,Fe^{2+} + 2\,H^+$$

The reducing effect of ascorbic acid in fact accelerates the oxidation reactions that lead to undesirable changes in flavour and colour of foods. These changes are the result of subsequent reaction of ions Fe^{2+} (and Cu^+) with oxygen that yields a radical of superoxide anion ($O_2^{\bullet-}$),[11] which further oxidises Fe^{2+} or Cu^+ and gives hydrogen peroxide. Further oxidation of Fe^{2+} or Cu^+ ions with hydrogen peroxide (Fenton reaction) generates hydroxyl radicals ($HO^\bullet$), which are the most important oxidants in biological systems (resulting in many pathological processes) and in foods:

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet-}$$
$$Fe^{2+} + O_2^{\bullet-} + H_2O + H^+ \rightarrow Fe^{3+} + HO^- + H_2O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet$$

Hydrogen peroxide can be replaced in these reactions by a fatty acid hydroperoxide (ROOH). Then, instead of hydroxyl radicals, alkoxyl radicals ($RO^\bullet$) are formed.

The prooxidant effects of ascorbic acid do not manifest when its concentration is sufficiently high, even though the increase of its concentration produces a higher amount of Fe^{2+} ions and hence $HO^\bullet$ radicals. In this case, a competitive reaction with free radicals takes place at the same time and ascorbic acid acts as an antioxidant.

5.14.6.1.4 Reaction with free radicals

Ascorbic acid, its isomers and derivatives can react with free radicals that cause oxidation of lipids and other oxylabile food components. It inhibits the radical chain autoxidation reaction and effectively acts as an antioxidant. The reaction of ascorbic acid with lipid (fatty acid) peroxyl radical ($ROO^\bullet$) or with alkoxyl radical ($RO^\bullet$) can be schematically represented by the following equation (ROOH is a fatty acid hydroperoxide):

$$H_2A + ROO^\bullet \rightarrow HA^\bullet + ROOH$$

The resulting ascorbyl radical ($HA^\bullet$) is no longer able to initiate a radical chain reaction and disproportionates into ascorbic acid and dehydroascorbic acid.

Ascorbic acid is generally a more effective antioxidant, when used in combination with tocopherols. They then preferentially react with lipid free radicals and the resulting tocopheryl radicals are, on the oil–water interface, reduced back to tocopherols by ascorbic acid. Ascorbyl palmitate yields, via the corresponding radical, dehydroascorbyl palmitate, which, unlike dehydroascorbic acid, cannot form a cyclic hydrate. Ascorbic acid also reacts similarly with toxic forms of oxygen, such as a hydroxyl radical ($HO^\bullet$), an anion of superoxide radical ($O_2^{\bullet-}$) and singlet oxygen ($^1O_2$). Simultaneously, all of these reactions slow down the oxidation of lipids:

$$H_2A + HO^\bullet \rightarrow HA^\bullet + H_2O$$
$$H_2A + O_2^{\bullet-} + H^+ \rightarrow HA^\bullet + H_2O_2$$

5.14.6.1.5 Acid catalysed degradation

In strongly acidic solutions, ascorbic acid can decarboxylate and, like other sugars, dehydrate. In model experiments almost quantitative amounts of carbon dioxide and furan-2-carbaldehyde are produced. The reaction mechanism is shown in Figure 5.30. An important product is 3-deoxy-L-*threo*-pentos-2-ulose, also known by its trivial name as 3-deoxy-L-xylosone, which plays an important role in the Maillard reaction of pentoses.

In the absence of atmospheric oxygen, acid-catalysed degradation of ascorbic acid is considered a major cause of the loss of vitamin C in dried fruits, canned fruit compotes and juices (pH value around 3.5), especially when stored at higher temperatures. For example, fruit juices lose 70–95% of ascorbic acid within 12 weeks of storage at 50 °C. The reaction rate is about ten times lower than that of autoxidation catalysed by metal ions.

5.14.6.1.6 Reaction with other food components

The loss of vitamin C may also occur during reactions of ascorbic acid with some of the reactive food components. In particular, reactions of ascorbic acid with quinones generated by enzymatic browning reactions, reactions with nitrites and haem pigments in meat and meat products are technologically significant.

Reactions with oxidised phenols

The enzymatic browning reactions of fruits and vegetables occur especially in materials that have a low content of ascorbic acid and active enzymes belonging to the group of *o*-diphenol:O_2 oxidoreductases. In damaged plant tissues and in the presence of atmospheric oxygen, these enzymes catalyse the oxidation of phenolic substrates (so-called monophenols or *o*-diphenols) to *o*-quinones, which then form brown polymeric pigments. As long

[11] Sometimes it is stated that instead of an anion of a superoxide radical, a hydrogen superoxide radical (it is actually a radical of hydrogen peroxide) forms and is in equilibrium with $O_2^{\bullet-}$ ($pK_a = 4.8$). The relevant equations then look as follows:

$$Fe^{2+} + O_2 + H_2O \rightarrow Fe^{3+} + HO_2^\bullet + HO^\bullet \quad \text{and}$$
$$Fe^{2+} + H_2O + HO_2^\bullet \rightarrow Fe^{3+} + HO^- + H_2O_2$$

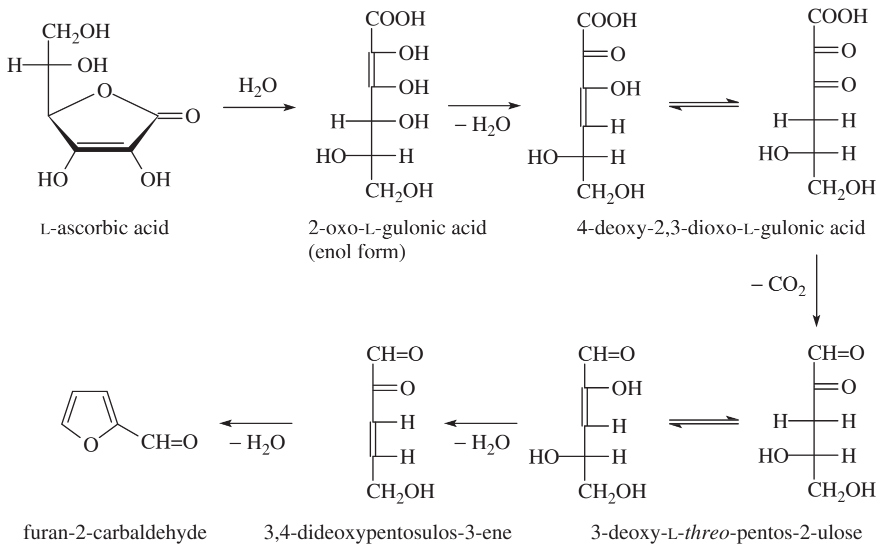
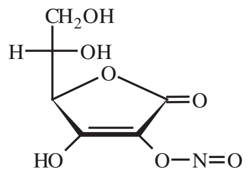


Figure 5.30 Degradation of L-ascorbic acid in acidic solutions.

as ascorbic acid is present, quinones are reduced back to the parent diphenols and an equimolar amount of dehydroascorbic acid is formed (see Section 9.12).

Reaction with nitrites and haem pigments

The disadvantage of the use of nitrites in meat manufacturing processes is the formation of toxic nitroso compounds (mainly *N*-nitrosamines) by reaction of nitrous acid (nitrite) with naturally present secondary amines (see Section 12.2.7.3.1). Ascorbic acid added together with nitrites decomposes the excess of nitrous acid, which is a precursor of nitrosation agents (primarily dinitrogen trioxide, N_2O_3). It is assumed that the reaction of ascorbic acid with nitrous acid temporarily creates the corresponding 2-ester (**5-124**), which is decomposed to an ascorbyl radical and nitric oxide (nitrogen monoxide, NO) that reacts with haem pigments. Ascorbic acid 2,3-diester has also been proposed as an intermediate, which decomposes to dehydroascorbic acid and nitric oxide. Minor products generated from nitrite (with myoglobin acts as a reducing agent) are nitrous oxide (N_2O) and nitrogen.



5-124, L-ascorbic acid 2-ester with nitrous acid

Even under physiological conditions, about 1% of haemoglobin in erythrocytes is present in the form of methaemoglobin, which is reduced to haemoglobin by methaemoglobin reductase. Myoglobin (Mb) is present in meat in the form of oxymyoglobin (MbO_2) and metmyoglobin (MetMb). Added ascorbic acid reduces both of these pigments to myoglobin (see Section 9.2.1.5.3). In the presence of ascorbic acid, a higher amount of myoglobin is available for the reaction, which creates the desired dye nitroxymyoglobin:

$$H_2A + MbO_2 + H_2O + e^- \rightarrow HA^{\bullet -} + MetMb + H_2O_2$$
$$H_2A + MetMb + e^- \rightarrow HA^{\bullet -} + Mb$$

Reaction with proteins

The aim of the Chorleywood bread process (see Section 5.14.4.4) is to use cheaper, lower-protein wheat and to reduce processing time. Flour, water, yeast, salt, fat, ascorbic acid and minor ingredients (emulsifiers and enzymes) are mechanically mixed, which results in rapid oxidation of ascorbic acid to dehydroascorbic acid, caused mainly by ascorbase activity in the dough that is a co-substrate of glutathione dehydrogenase. Glutathione dehydrogenase oxidises reduced glutathione (G-SH) to the corresponding disulfide (oxidised glutathione, G-S-S-G), which does not affect the rheological properties of dough:

$$A + 2G\text{-}SH \rightarrow H_2A + G\text{-}S\text{-}S\text{-}G$$

The content of reduced glutathione in wheat flour is 10–15 mg/kg. In the absence of ascorbic acid (dehydroascorbic acid, respectively), G-SH reacts with components of gluten (P) that are linked by disulfide bridges (P-S-S-P) to form mixed disulfides, which show negative effects on the baking properties of flour:

$$P\text{-}S\text{-}S\text{-}P + G\text{-}SH \rightarrow P\text{-}S\text{-}S\text{-}G + P\text{-}SH$$

Free cysteine (Cys-SH) formed by hydrolysis of glutathione can also depolymerise gluten proteins or react with mixed disulfides that also have negative effects on the baking properties of flour:

$$P\text{-}S\text{-}S\text{-}Cys + G\text{-}SH \rightarrow P\text{-}S\text{-}S\text{-}G + Cys\text{-}SH$$
$$P\text{-}S\text{-}S\text{-}P + Cys\text{-}SH \rightarrow P\text{-}S\text{-}S\text{-}Cys + P\text{-}SH$$
$$P\text{-}S\text{-}S\text{-}G + Cys\text{-}SH \rightarrow P\text{-}S\text{-}S\text{-}Cys + G\text{-}SH$$

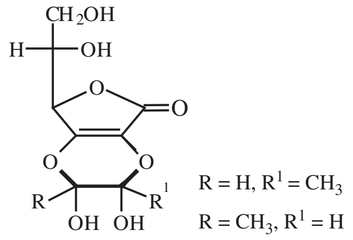
Reaction with glucosinolate degradation products

Ascorbic acid carbanion (C-2) reacts with electrofilic agents (carbonium cations). A typical example of this reaction is the formation of ascorbigen in the reaction of ascorbic acid with the degradation products of indole glucosinolates in Brassica vegetables. The glucosinolate glucobrassicin (and its analogues) is hydrolysed to glucose, hydrogensulfate anion and unstable isothiocyanate by the enzyme myrosinase (thioglucosid glucohydrolase) in the damaged plant tissue (such as shredded cabbage). The anion of the tautomeric form of ascorbate then replaces thiocyanate (rhodanide) anion in this intermediate, which yields ascorbigen (see Section 10.3.2.4.2). Ascorbigen is more stable to oxidation than ascorbic acid and exhibits anticarcinogenic effects.

Another ascorbic acid derivative is a water-soluble tannin called elaeocarpusin, which was isolated from the leaves of the evergreen tropical tree *Elaeocarpus sylvestris* (Elaeocarpaceae). Elaeocarpusin is derived by condensation of corilagin (see Section 8.3.5.1.2, Figure 8-294) with dehydroascorbic acid.

Reaction with carbonyl compounds

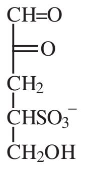
The reaction of ascorbic acid with aldehydes in aqueous solutions yields the corresponding hemiacetals. The reaction with methylglyoxal (a typical product of carbohydrate degradation) gives rise to two cyclic hemiacetals (**5-125**). Similar products are produced with acrolein, the degradation product of glycerol.



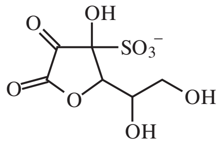
5-125, L-ascorbic acid acetals with methylglyoxal

Reaction with sulfur dioxide

Sulfur dioxide reduces dehydroascorbic acid to ascorbic acid and is therefore frequently used in combination with ascorbic acid, particularly in fruits and vegetables. It has been suggested that under anaerobic conditions, sulfite ion may catalyse degradation of ascorbic acid and readily adds across the double bond of the 3,4-dideoxypentosulos-3-ene (Figure 5.30) that is formed to yield 3,4-dideoxy-4-sulfopentos-2-ulose (**5-126**). Under aerobic conditions, the bisulfite ion forms hydroxysulfonate of dehydroascorbic acid (**5-127**).



5-126, 3,4-dideoxy-4-sulfopentos-2-ulose



5-127, hydroxysulfonate of dehydroascorbic acid

5.14.6.2 Dehydroascorbic acid

Like other vicinal dicarbonyl derivatives of sugars, dehydroascorbic acid is involved in the Maillard reaction. Dehydroascorbic acid (or its bicyclic hydrate, Figure 5.27) is a γ-lactone that is readily hydrolysed under a base catalysis to its parent unstable compound that undergoes a series of irreversible reactions. These reactions result in loss of vitamin C and the formation of coloured products, and the discoloration of fruit and vegetable products.

5.14.6.2.1 Hydrolysis

Dehydroascorbic acid hydrolysis yields biologically inactive 2,3-dioxogulonic (L-*threo*-hexo-2,3-diulosonic) acid. In aqueous solutions, this acid is present as a dihydrate (Figure 5.31). The reaction is generally acid-base catalysed. The activity of important ions and undissociated molecules decreases in the order: hydroxyl ions (HO^-) > hydronium ions (H_3O^+) > anions of carboxylic acids $R{-}COO^-$) > undissociated carboxylic acids (R–COOH) > water. The catalytic effect of hydroxyl ions is about 15×10^6 times higher than that of hydronium ions. This means that the reaction rate in the solution of pH 4 is 15×10^6 times lower than in solution of pH 10. Dehydroascorbic acid is most stable in solutions of pH 2.5–5.5, where the reaction is only catalysed by undissociated water molecules and is rapidly hydrolysed in neutral and alkaline solutions.

5.14.6.2.2 Subsequent reactions

2,3-Dioxogulonic acid is an unstable compound and undergoes a series of reactions that are analogous to reactions of sugars. Its decomposition produces many products depending on the pH and the presence of atmospheric oxygen. In acidic solutions, the main transformation products are furan derivatives, decomposition in alkaline solutions produces organic acids (their salts) and reductones. The key reaction that occurs in a wide range of pH values (in acidic and alkaline solutions) is decarboxylation to (3*R*,4*S*)-3,4,5-trihydroxy-2-oxopentanal (L-*threo*-pentosulose). In older literature this substance was called L-xylosone and its enolform was known as reductone C. It is assumed that this enolform reacts with another molecule of 2,3-dioxo-L-gulonic acid, which is reduced to L-ascorbic acid, while L-*threo*-pentosulose is simultaneously oxidised to L-erythroascorbic acid, also known as (*S*)-2,3,4,5-tetrahydroxypent-2-enonic acid (Figure 5.31). Some other reactions of L-*threo*-pentosulose are shown in Figure 5.32.

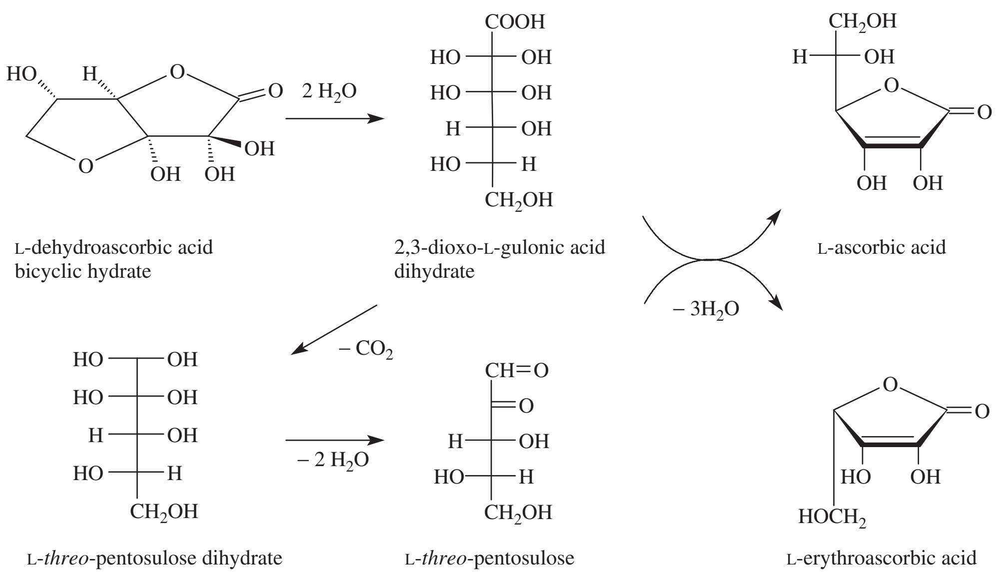


Figure 5.31 Degradation of L-dehydroascorbic acid.

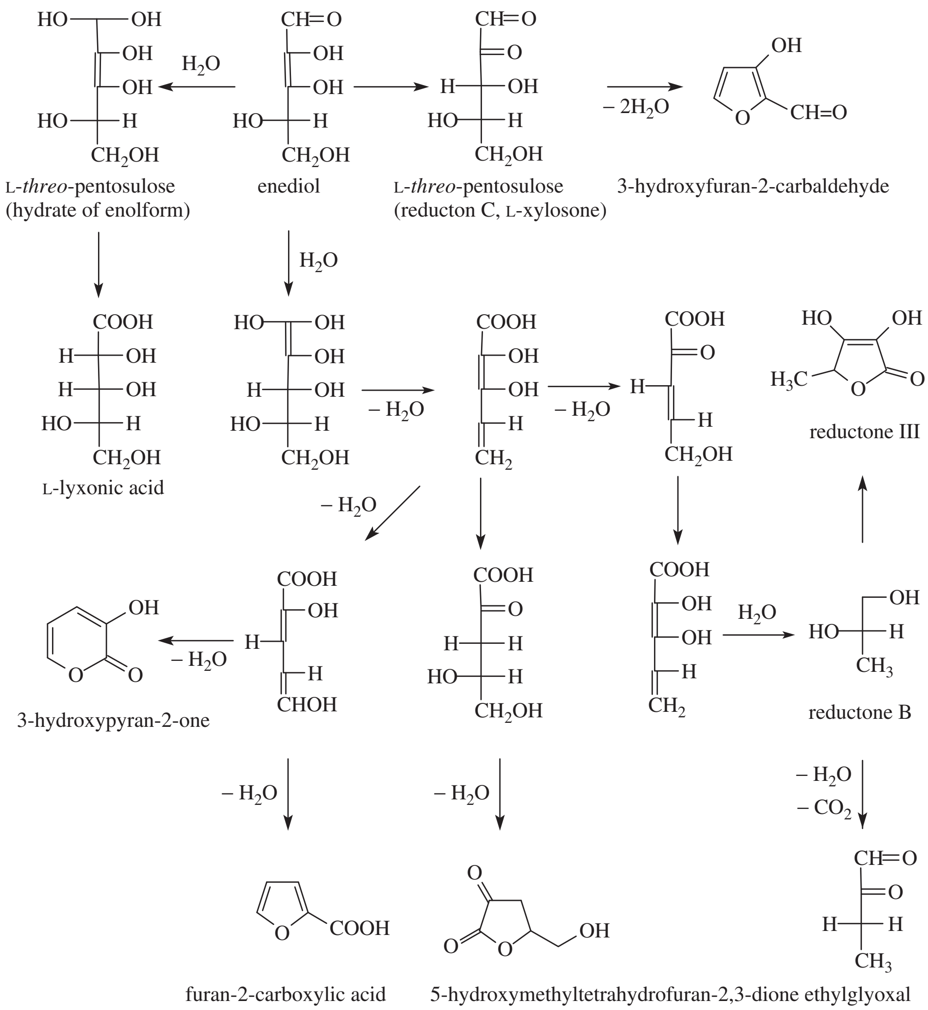
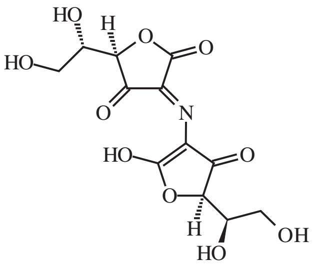


Figure 5.32 Degradation of L-*threo*-pentosulose.

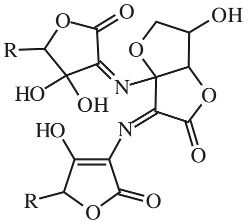
The main products formed by a sequence of isomerisation and (de)hydration reactions are 3-hydroxypyran-2-one and furan-2-carboxylic acid (also known as pyromucic or 2-furoic acid). The odour of 3-hydroxypyran-2-one is reminiscent of caramel (maltol and isomaltol have similar odours).

5.14.6.2.3 Reactions with amino compounds

Dehydroascorbic acid reacts with amines, amino acids and proteins and participates in the non-enzymatic browning reactions that occur mainly in foods with low water activity and relatively high content of vitamin C (e.g. in dehydrated fruit juices). The primary product of the reaction with amino compounds is imine. Reduction of imine gives the corresponding amine. Isomerisation of imine and elimination of carbon dioxide and aldehyde yields scorbamic acid (Figure 5.33). This reaction is known as Strecker degradation of amino acids. A detailed reaction mechanism is given in Section 2.5.2.3. This 2-amino derivative reacts with another molecule of dehydroascorbic acid and yields the so-called red pigment (**5-128**). Formation of this pigment, which sometimes occurs during the preservation of cauliflower, leads to unwanted discoloration of the preserved product. The subsequent reaction of scorbamic acid with the red pigment creates the yellow pigment (**5-129**), which is probably an intermediate in reactions leading

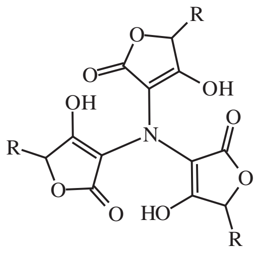


5-128, red pigment of ascorbic acid



5-129, yellow pigment of ascorbic acid

from dehydroascorbic acid to brown polymeric pigments called melanoidins. Other reaction products include cyclic nitrogenous products, and typically unstable free radicals and stable products, such as tris(2-deoxy-2-L-ascorbyl)amine (**5-130**).



5-130, tris(2-deoxy-2-L-ascorbyl)amine

5.14.7 Changes in foods

Ascorbic acid is one of the least stable vitamins. It is sensitive to heat, light and oxygen. Storage of foods, cooking procedures and industrial processing methods can result in significant loss of vitamin C. The most significant losses are caused by vitamin leaching and oxidation. In the absence of atmospheric oxygen, losses are mainly caused by acid catalysed degradation. Total losses generally range between 20 and 80%.

5.14.7.1 Fruits and vegetables

If fresh products are held at the appropriate temperature and consumed in a short period of time, they have more vitamin C than commercially canned products. Vitamin C degrades rapidly after harvest and its amount depends on the commodity, temperature, pH and other factors. During storage (and processing), the stability of ascorbic acid is higher in fruits that have lower pH than vegetables. For example, the effect of storage on the vitamin C content in

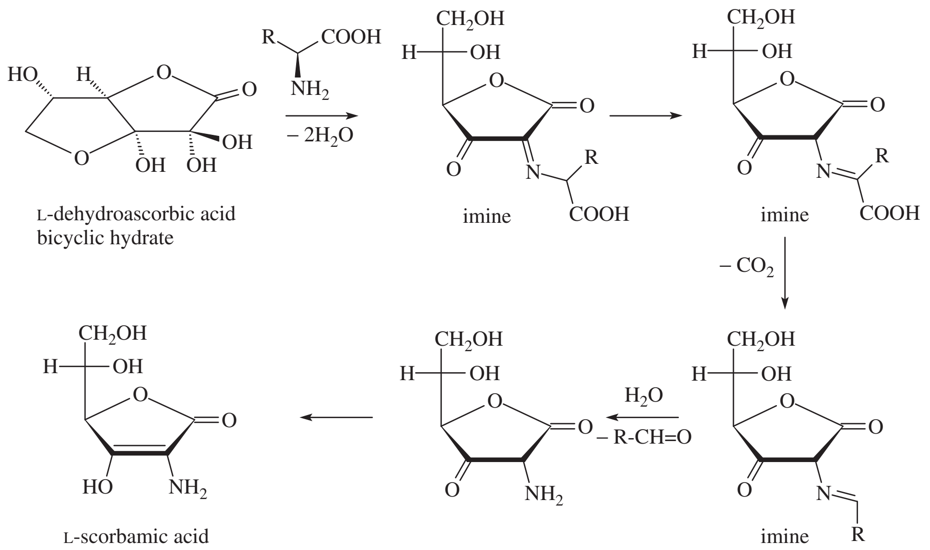


Figure 5.33 Formation of L-scorbamic acid from L-dehydroascorbic acid and amino acids.

apples depends upon the time of picking, storage temperature and composition of the storage atmosphere. Apples (Golden Delicious variety) stored at 25 °C lose 41% of vitamin C in 2 weeks after harvesting and almost 56% is lost after 6 weeks of storage. Only small changes in the vitamin C content occur at 4 °C. Green beans, however, lose up to 77% of vitamin C in 7 days of storage at 4 °C. The vitamin C level in fresh potatoes falls to 30–60% of the original level in the first 2 months after harvest, but then tends to stabilise at 25% of the original level.

Losses of ascorbic acid due to leaching occur during washing, blanching, cooking and preserving fruits and vegetables in cases where the extract is not further processed. Losses during washing are lower than losses during blanching and cooking. The nature and extent of losses depends on pH, temperature, water quantity, surface area that is in contact with water, maturity, extent of contamination by metals and presence of oxygen. A significant decrease in vitamin C content is also caused by peeling the fruit, when the surface layer rich in vitamin C is removed.

The average retention of fruit juices fortified with vitamin C is 60–80%. Between 10 and 90% of vitamin C is lost during canning of fruits, however the changes are little during storage of canned products and little is lost during re-heating, because the heating time is short. The lowest losses are achieved during short-term sterilisation at high temperature. In fruit treated with sulfur dioxide, the losses of ascorbic acid during technological processing are lower as sulfur dioxide reduces hydrogen peroxide produced by oxidation of ascorbic acid in the presence of heavy metals. Vitamin C is most stable during the freezing and refrigerated storage of fruits and vegetables. Storage at temperatures of −18 °C result in only minimal losses, while significant losses can occur during thawing (30–50%).

Losses are higher in green leafy vegetables with a large surface than in root vegetables. Degradation of vitamin C also occurs during processing of potatoes with absolute losses in the order of 30%, although the concentration of vitamin C in potato crisps can be higher than in fresh potato due to a reduction of water content during frying. Vitamin loss also occurs during fermentation of vegetables. Sauerkraut, for example, contains about 50% of the vitamin as compared with fresh cabbage (90–190 mg/kg).

The best methods for small-scale processing are drying, chemical preservation and heat processing; these and other methods may be applied in industrial processing. In order to retain the maximum amount of vitamin C in fruits and vegetables during storage and in processed products (based on reaction mechanisms described above), the following principles should be maintained:

- Fruits and vegetables should be used when freshly harvested.
- They must not be subjected to long soaking, washing or blanching.
- They must be processed immediately after preparation.
- The level of metal ions should be reduced by exclusion of any direct contact with copper, bronze, brass and iron parts of processing equipment or copper, iron or chipped pans; binding of metal ions to inactive complexes by chelating agents (such as ethylenediaminetetraacetic acid, or EDTA, citrates and phosphates); autoxidation of ascorbic acid is also slower in the presence of proteins, acidic polysaccharides and flavonoids; unfavourable conditions for the formation of complexes of metal ions with ascorbic acid may be created by lowering water activity, pH value, using appropriate *O*-2 substituted derivatives of ascorbic acid, such as 2-phosphate or 2-*O*-α-D-glucoside.
- Contact with air should be minimised by reducing the amount of oxygen (reduced pressure, inert atmosphere, addition of hydrogen bisulfites, fermentation, use of glucose oxidase and catalase; glucose oxidase catalyses the oxidation of D-glucose by oxygen to D-gluconic acid and hydrogen peroxide, which is then reduced by catalase to water:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

5.14.7.2 Milk and dairy products

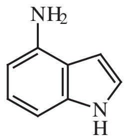
Losses of ascorbic acid during storage of raw milk are considerable. Cold storage causes about 50% loss of vitamin C, which increases with increased temperature. Heat treatment of milk decreases the content of vitamin C by 20–50%, depending on temperature and time of heating. The UHT treatment of milk causes about 10–30% loss. Ascorbic acid in dried, vitamin-enriched milk, packaged in an inert atmosphere, is relatively stable.

5.15 Other active substances

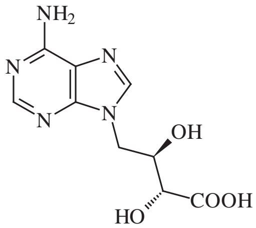
In the past, vitamins have included far more substances than today. The catalytic effect of some hydrophilic substances was subsequently not reliably proven in the metabolism of humans (such as 4-aminobenzoic acid, the building unit of folacin). The human body can synthesise some compounds in adequate amounts, such as adenine (vitamin B_4), 4-aminobenzoic acid (vitamin B_{10}), orotic acid (vitamin B_{13}), carnitine (vitamin B_{20}), *myo*-inositol (vitamin B_m), choline (vitamin B_p), 2-aminobenzoic (anthranilic) acid previously known as vitamin L_1, 9′-(5-thiomethylribofuranosyl)adenine, which is a metabolite of RNA previously known as vitamin L_2, and salicylic acid, previously known as vitamin S. Furthermore, no signs of their essentiality and avitaminosis have been demonstrated. The term vitamin never included other essential nutrients such as thioctic acid, taurine, coenzyme Q and other biologically active compounds. Some substances are vitamins only for microorganisms (4-aminobenzoic acid, thioctic acid and other growth factors of microorganisms). A range of biologically active compounds initially ranked among the vitamins are now categorised among other substances and are no longer considered true vitamins, such as essential fatty acids (previously known as vitamin or vitagen F) are classified as lipid constituents, bioflavonoids (previously vitamin P) are plant pigments called flavonoids, while vitamin J was a mixture of non-essential catechol and riboflavin. Coenzyme Q, carnitine, taurine, orotic acid, *myo*-inositol, choline and other compounds

are currently used as food supplements.[12] Some of the claimed benefits of food supplements have a more scientific basis than others, but all must be carefully evaluated and the supplemented compounds should not replace a varied and balanced diet.

The purine base adenine (6-aminopurine, previoulsy also known as vitamin B_4, **5-131**) is generally widespread as a building unit of the ribofuranoside adenosine and its phosphates, such as adenosine 5′-monophosphate (AMP, adenylic acid), adenosine 5′-diphosphate (ADP) and adenosine 5′-triphosphate (ATP), polynucleotides, such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), vitamins (riboflavin, adenosylvitamin B_{12}) and cofactors $NAD(P)^+$ and FAD. Adenylic acid was once considered a member of the vitamin B complex (as vitamin B_8). The agent in shiitake (*Lentinula edodes*) mushrooms that can reduce the level of cholesterol in the blood is a purine derivative, (2*R*,3*R*)-dihydroxy-4-(9-adenyl)butyric acid (**5-132**) called eritadenine (lentinacin, lentysine).

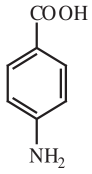


5-131, adenine

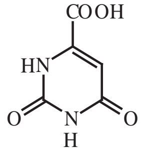


5-132, eritadenine

4-Aminobenzoic acid, also known as *p*-aminobenzoic acid (PABA), was previously ranked among the B group vitamins as vitamin B_{10}, vitamin B_x or vitamin H_1 (**5-133**). It arises from chorismic acid and commonly occurs in foods of animal and plant origin. Good sources include organ meats, such as liver and kidney, eggs, whole wheat flour, wheat germ and brewer's yeast. Aminobenzoic acid is also supplied by intestinal microorganisms. Aminobenzoic acid is probably most important as an intermediate in the biosynthesis of folacin; therefore it is a growth factor of some bacteria, but not for humans. Despite the lack of any recognised syndromes of aminobenzoic acid deficiency in humans, many claims of benefits are made by commercial suppliers of aminobenzoic acid as a nutritional supplement. Aminobenzoic acid supposedly participates in many metabolic processes in humans. It appears to function as a coenzyme in the conversion of certain chemical intermediates into purines. It has also been suggested that it has an antifibrosis activity and increases oxygen uptake at the tissues. These effects are, at present, still considered speculative. When it is in short supply, fatigue, irritability, nervousness and depression might manifest itself as well as constipation. Weeping eczema has also been noted in people with 4-aminobenzoic acid deficiency, as well as patchy areas on the skin. Aminobenzoic acid physically blocks ultraviolet rays when it is applied to the skin (it is a UVB absorber) and is sometimes suggested for treatment various skin diseases, such as weeping eczema (moist eczema), scleroderma (premature hardening of skin), depigmentation of skin (vitiligo) and premature grey hair, as well as for male infertility. The recommended daily allowance is 0.05 mg for infants, 0.2–0.3 mg for children, 0.4 mg for adults, 0.8 mg for pregnant women and 0.6 mg for lactating women.



5-133, 4-aminobenzoic acid



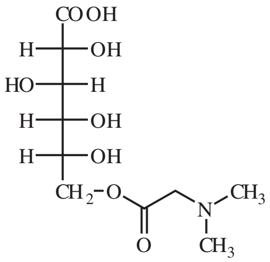
5-134, orotic acid

Orotic acid (originally known as vitamin B_{13}, **5-134**) is an intermediate in the biosynthesis of pyrimidines required for DNA and RNA synthesis, but essentiality has not been demonstrated. It is synthesised from carbamoyl phosphate and aspartic acid through dihydroorotic acid and then transformed into orotidin 5′-phosphate and further to uridine 5′-monophosphate. When there is insufficient capacity to detoxify the ammonia load for urea synthesis, carbamoyl phosphate leaves the mitochondria and enters the pyrimidine pathway, where orotic acid biosynthesis is stimulated; orotic acid excretion in urine then increases. Orotic acid synthesis is abnormally high in hereditary deficiencies of urea-cycle enzymes or uridine monophosphate synthase. This acid occurs mainly in milk from ruminants. In cows' milk, the level is 20–100 mg/l and in somewhat higher amounts are found in goats' and sheeps' milk (200–400 mg/l). In human milk, concentrations are in general low (under 2 mg/l). Good sources of orotic acid are also dairy products (at a level of about 150 mg/l in yoghurt), liver and baker's yeast. The benefit claims reportedly include the enhanced formation of albumen in the liver, especially in conditions of prolonged hypoxia that occurs in some diseases, such as heart failure and has a positive effect on foetal development during pregnancy. The effectiveness of orotic acid was shown in children from 6 months to 10 years, suffering from various skin diseases (eczema, atopic dermatitis, psoriasis and ichthyosis). The daily dose suggested by commercial suppliers is 0.125–0.5 g for children 1–3 years old, 0.25–1 g for children 3–8 years old and 0.5–1.5 g, or sometimes up to 3 g, for adults.

Pangamic acid (formerly known as vitamin B_{15}, **5-135**) occurs mainly in cereals, legumes, seeds (sunflower and pumpkin seeds) and yeast. Its nutritional value is debatable and no scientific evidence exists to substantiate any physiological function or biological effects. It probably acts (like choline) as a lipotropic factor, which stimulates

[12] Some EU member states (e.g. the Czech Republic in Decree No. 225/2008 Coll.) have set maximum permissible amount in daily intake for orotic acid (50 mg), acetylcarnitine (500 mg), and taurine (200 mg). The use of orotic acid salts as a source of minerals and choline is a safety concern.

the formation of very low density lipoproteins (VLDL) in the liver. Although no evidence supports its use, pangamic acid has been used to treat cancer, schizophrenia, *diabetes mellitus*, heart diseases, alcoholism, hepatitis and indigestion. It has also been asserted that pangamic acid detoxifies byproducts of human metabolism.



5-135, pangamic acid

N,*N*-Dimethylglycine (originally called vitamin B_{16}; see Section 2.2.1.2.1) is an amino acid found in almost all animal and plant cells and as a building unit of pangamic acid. It can be formed by demethylation of *N*,*N*,*N*-trimethylglycine (glycine betaine), a very efficient cytoplasmic osmolyte of some plants and bacteria or as an intermediate in the pathway leading from choline to glycine. Demethylation of dimethylglycine yields *N*-methylglycine (sarcosine). Dimethylglycine is contained in higher amounts in certain foods including liver, beans, cereal grains, many seeds and brewer's yeast. Deficiencies in dimethylglycine do not cause any adverse effects. Manufacturers of dimethylglycine supplements claim that as a supplement, vitamin B_{16} can improve athletic performance (it assists in oxygen utilisation within the body), enhances the immune system, stimulates neurological functions, helps to manage autism (noticeable improvements in cognitive functions are exhibited in combination with vitamin B_6 supplementation) and epilepsy and provides protection of cells against free radicals as an antioxidant.

The cyanogenic glycoside known as (*R*)-amygdalin, a β-gentiobioside of D-mandelic acid nitrile (see Section 10.3.2.3.1), is sometimes incorrectly referred to as vitamin B_{17}. Amygdalin is often confused with semi-synthetic laevomandelonitrile, also called laetrile for short, which is a β-glycoside of D-mandelic acid nitrile with D-glucuronic acid. Amygdalin occurs in plant seeds of the rose family (Rosaceae). Significant resources are bitter almonds and the pits of apricots, peaches, plums and cherries. Small amounts of amydalin are also present in the cores of apples, pears, quinces and other plant seeds. Amygdalin is capable of decomposing into a sugar molecule(s), benzaldehyde and toxic hydrogen cyanide through the action of the hydrolase amygdalase (also called emulsin). The enzyme rhodanase (in bacteria, plants and animals) acts in the catabolism of cyanides (R–C≡N) catalysing conversion of cyanides into thiocyanates (rhodanides, R–S–C≡N). Perhaps the most notable (yet controversial) benefit of amygdalin is its effectiveness in treating cancer, in particular prostate cancer, arthritic pain and high blood pressure. The claimed anticarcinogenic activity is based upon the fact that cancer tissues are rich in hydrolase that causes amygdalin to release cyanide, which destroys the cancer cells. According to this theory, non-cancerous tissues are protected from this fate by active rhodanase, which renders the cyanide harmless. However, no substantive benefit was observed in terms of cure, improvement or stabilisation of cancer, improvement of symptoms related to cancer or extension of life span. Furthermore, the hazards of amygdalin therapy were evidenced in several patients by symptoms of cyanide toxicity.

L-Carnitine, also known as (*R*)-carnitine or trimethylammonium-3-hydroxybutyrobetaine (formerly known as vitamin B_{20}, vitamin O or vitamin B_t; see Section 2.2.1.2.1) is synthesised from lysine and methionine in many organisms, ranging from bacteria to mammals. The lysine becomes available in the form of ε-(*N*,*N*,*N*-trimethyl)lysine after lysosomal hydrolysis of proteins that contain this amino acid as a result of the post-translational methylation of lysine residues. Carnitine, in the activated form as acylcarnitine, serves as a carrier of fatty acid residues across the inner membrane of the mitochondria, which helps in the consumption and disposal of fat in the body. Then, fatty acids can be degraded by β-oxidation to acetyl-CoA to obtain energy via the citric acid cycle. The highest amount of carnitine is present in the muscles of animals, in red meat levels range from 0.05 to 0.2% fresh weight. Good sources are also poultry and fish. Carnitine (free carnitine, acetylcarnitine or propionylcarnitine) is often sold as a nutritional supplement for stimulation of energy metabolism of fatty acids, which is used in weight reduction and also in sports nutrition. Although carnitine has been marketed as a weight loss supplement, there is no scientific evidence to show that it works. Some studies do show that oral carnitine reduces fat mass, increases muscle mass and reduces fatigue, which may contribute to weight loss in some people. Some research suggests that carnitine may help prevent or reduce symptoms of an overactive thyroid gland, slow down the progression of Alzheimer's disease, relieve depression related to senility and other forms of dementia and improve memory in the elderly, improve male sexual function and increase sperm count and mobility. Recommended doses of carnitine for adults vary depending on the health condition being treated. The usual dose is between 1 and 3 g/day.

The cyclic hexitol *myo*-inositol (incorrectly also known as *meso*-inositol; see Section 4.3.1.1.2) has previously been considered as one of the B group vitamins (called i-inositol, phaseomannitol, nucitol, vitamin B_m or bios I). Inositol is synthesised by conversion of D-glucose 6-phosphate and plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells, including inositol phosphates and phosphatidylinositol in phospholipids. It is found in many foods, in particular, in legumes, cereals with high bran content, nuts and fruits. Concentrations of inositol in legumes range from about 200 to 3000 mg/kg dry weight. Phosphatidylinositol (and phosphatidylcholine) show lipotropic effects and prevent fatty liver disease, such as abnormal deposition of lipids within liver cells (steatosis). Inositol is a growth factor for various microorganisms. Deficiency in animals manifests by hair greying and falling out, but specific avitaminosis was not observed in humans. The manufactures of food supplements claim that inositol may be beneficial for treating certain psychiatric conditions, such as obsessive-compulsive disorder, panic attacks, trichotillomania (an impulse control disorder) and bipolar disorder. In addition, inositol may be useful for ameliorating some of the symptoms of polycystic

ovary syndrome (a female endocrine disorder). The daily intake of *myo*-inositol is estimated to be 1000 mg. The recommended daily dose from food supplements is 500 mg.

In plants (mainly in cereals and legumes), inositol occurs in a bound form known as phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphoric acid). Salts of this acid are called phytates. Calcium-magnesium salt of phytic acid, which also contains a number of other mineral elements, is called phytin (see Section 6.2.2.3). Phytic acid plays a role as an antioxidant (due to strong binding of transition metals) and anticarcinogenic effects have also been demonstrated. Partial esters of phytic acid are used to regulate the amount of calcium in cells, exhibit anti-coagulant, anti-inflammatory and other effects. The other bound form of *myo*-inositol with sugar derivatives are pseudooligosaccharides (see Section 4.3.1.2.2) that occur in legumes.

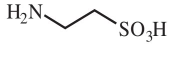
Previous classification ranked choline, *N*,*N*,*N*-trimethylethanolammonium cation (with chloride or hydroxyl group as a counter ion), amongst the vitamins of the B group, and it was known as vitamin B_p (see Section 3.5.1.1.1). Plants and animals synthesise choline from glycine or serine, an intermediate of the biosynthesis is ethanolamine, which is methylated by folacin to yield choline. Choline is used for biosynthesis of phospholipids (phosphatidylcholine and sphingomyelin) occurring in cell membranes, in the biosynthesis of acetylcholine acting in the transmission of nerve impulses, participates in the transmethylation processes in the organism via its metabolite *N*,*N*,*N*-trimethylglycine (glycine betaine) and is a precursor of trimethylamine. Persons with the genetic disorder trimethylaminuria (the inability to metabolise trimethylamine) suffer from an unpleasant fishy body odour. Important food sources of choline include butter, egg yolk, pulses, oilseeds, nuts and cereals. The daily choline requirement is around 600 mg. Mild deficiency has been linked to fatigue, insomnia, poor ability of the kidneys to concentrate urine, problems with memory and nerve-muscle imbalances. Extreme dietary deficiency can result in liver dysfunction, cardiovascular disease, impaired growth, kidney failure, high blood pressure and other symptoms. Soybean lecithin is the most common form of supplemental choline, but choline itself is also available in food supplements. It is used therapeutically (perorally and parenterally) in various liver diseases (e.g. steatosis and cirrhosis), for maintenance of cell membrane integrity, support of nervous system activity and to lessen chronic inflammation. Adequate intake levels established by the U.S. National Academy of Sciences are: 125 mg (0–6 months), 150 mg (6–12 months), 200 mg (1–3 years), 250 mg (4–8 years), 375 mg (males and females 9–13 years), 400 mg (females 14–18 years), 425 mg (females 19 years and older), 450 mg (pregnant females), 550 mg (males 14 years and older and lactating females). Prevention of liver damage was the main criterion used in establishment of these recommended levels.

Acetylcholine is found in foods of animal origin. In some plants, such as rape seed, choline is found in the form of various phenolic acid esters, especially in sinapic (4-hydroxy-3,5-dimethoxycinnamic) acid esters, which are trivially called sinapine (see Section 8.2.7.1.1).

Bioflavonoids, also known as vitamin P (the permeability vitamin) are a group of biologically active flavonoids (flavonol and flavanone glycosides) that affect the permeability and elasticity of blood capillaries. These effects were first observed in a compound known as citrine obtained from citrus fruits. Later it was found that citrine is a mixture of hesperidin and eriocitrin (also known as eriodyctin). Significant biological effects have been demonstrated mainly in quercetin-3-β-rutinoside known as rutin (see Section 9.4.2.4). Bioflavonoids are found mainly in fruits and vegetables at a level of about 100–7000 mg/kg.

Vitamin U, also known as *S*-methyl-L-methionine, methylmethionine sulfonium chloride, cabigen or antiulcer vitamin, was originally called vitamin U, because of its usefulness against ulceration of the digestive system. It is produced from methionine in an enzymatically catalysed methylation by *S*-adenosyl-L-methionine (SAM), but can also arise in non-enzymatic reactions, for example by methylation of methionine with pectin. *S*-Methylmethionine serves as the storage form of labile methyl groups in plants and plays a role in preventing accumulation of the methylation agent SAM. It occurs mainly in *Brassica* vegetables where it is a precursor of dimethyl sulfide during thermal processing. Its cabbage-like odour plays an important role in cooked vegetables. The content of *S*-methionine is about 90 mg/kg in kohlrabi, 75–81 mg/kg in cabbage, 124 mg/kg in turnip, cabbage and 60 mg/kg in Savoy cabbage and Brussel sprouts. High concentrations of *S*-methylmethionine are also found in celery (60–176 mg/kg), leeks (60–94 mg/kg) and beetroot (89 mg/kg), but its concentration in tomatoes is only 2.8 mg/kg. In other vegetables the levels are lower and those found in fruits are about 1 mg/kg. *S*-Methylmethionine was recommended for the therapy of peptic ulcers, particularly of duodenal ulcers and also in hyperlipidaemia. The traditional use of raw cabbage juice for the treatment of peptic ulcers would seem to support the use of vitamin U supplements as a healing aid for damaged and eroded intestinal mucous. There is currently no recommended daily allowance of vitamin U. The recommended daily dose from food supplement manufacturers is 500–1000 mg.

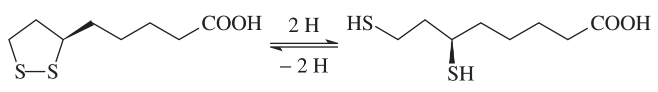
Taurine (1-aminoethane-2-sulfonic acid, **5-136**) is the only known naturally occurring sulfonic acid that is essential for many biological processes. The major pathway of taurine metabolism is the synthesis of bile acids, which emulsify dietary fat and promote its processing in the intestines. Taurine is further involved in calcium metabolism modulation, neuroinhibition in the central nervous system, reproduction, osmoregulation, as well as the anti-inflammatory activity of leukocytes and platelet aggregation. It also plays a significant role as an antioxidant preventing the oxidative damage that occurs during the aging process. Recent studies have provided evidence that taurine also becomes a constituent of some biological macromolecules. For example, taurine-containing modified uridines have been found in the human mitochondria. Taurine combined with higher fatty acids, such as 2-(octadecanoylamino)ethanesulfonic acid, occur in cells of some protozoa (such as *Tetrahymena thermophila*). Taurine is biosynthesised from cysteine by two main distinct pathways, via cysteine



5-136, taurine

sulfinic acid and hypotaurine and via cysteic acid. Hypotaurine can likewise be produced from L-cysteamine. In mammalian tissues, taurine is a ubiquitous semi-essential amino acid occurring as a free compound, although its concentrations in different tissues and fluids vary widely. It also occurs in insect tissues and it is particularly abundant in flight muscle and in eyes. On the other hand, taurine is completely absent in plants. The estimated mean daily intake of taurine in food is around 58 mg. Good sources of taurine include meat (0.02–0.1% fresh weight) and some types of seafood, the latter being particularly rich sources. In mammalian tissues, taurine is the most abundant amino acid in the skeletal muscle, heart, retina, brain and leukocytes. Taurine is used as an ingredient in many energy drinks and energy products, usually containing 4000 mg/l taurine. They are marketed worldwide for the treatment of various physiological conditions, for improvement of athletic performance and for general wellbeing. Taurine is also available in food supplements and is claimed to treat a wide range of conditions, from alcoholism and hepatitis to congestive heart failure. There is no recommended dietary allowance for this compound. The daily dose recommended by producers of dietary supplements varies between 2000 and 4000 mg.

The lipophilic sulfur-containing coenzyme (*R*)-lipoic (α-lipoic) acid has the systematic name (*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid. Lipoic acid is synthesised as an off-shoot of the fatty acid biosynthesis pathway in a wide number of organisms, including bacteria, fungi, plants and animals. Lipoic acid is essential for the activity of a variety of enzyme complexes that catalyse oxidative decarboxylation. In the cell, very little lipoic acid exists as the free acid; almost all is bound to the ε-amino group of the lysine residue of target complexes. In plants, lipoic acid as the prosthetic group of pyruvate oxidase is a key substance in photosynthesis and acts as a growth factor of many bacteria. Besides its activity as a coenzyme, lipoic acid is considered as an efficient antioxidant since, in its reduced form, (*R*)-dihydrolipoic acid (6,8-disulfanyloctanoic acid or 6,8-dimercaptooctanoic acid), it constitutes a redox couple via modulation of the NADH/NAD^+ ratio (Figure 5.34). Dihydrolipoic acid can scavenge hydroxyl and peroxyl radicals, but can also chelate transition metals (such as Fe and Cu). Lipoic acid occurs in almost all foods. Good sources are offal, yeast and some vegetables (such as spinach and broccoli), but lipoic acid is not readily available as it occurs in bound forms. Lipoic acid is also available in food supplements, primarily as an antioxidant and weight loss agent. It has also been suggested for treatment of cataracts, glaucoma, multiple sclerosis, burning mouth syndrome, Alzheimer's disease and strokes. The daily dose suggested by commercial suppliers is 200–300 mg, but no recommended daily allowance has been established.

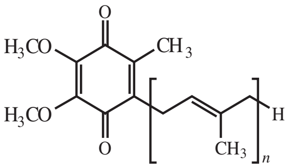


(*R*)-lipoic acid (*R*)-dihydrolipoic acid

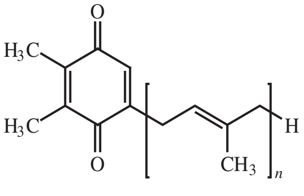
Figure 5.34 Reactions of lipoic acid.

A similar compound, 1,2-dithiacyclopentane-3-carboxylic acid (also known as tetranorlipoic or tetranorthioctic acid), was recently isolated from garlic.

Coenzymes Q_n (in short CoQ_n or ubiquinones-*n*) are 2,3-dimethoxy-5-methyl-1,4-benzoquinones with a long side-chain attached to C-2, which is composed of varying number of isoprene units ($n = 1–12$), which is dependent on the species (**5-137**). Most organisms synthesise a range of compounds, of which those where $n = 7–10$ usually predominate. Coenzyme Q_n functions as a mobile electron carrier within the mitochondrial inner membrane in all aerobic eukaryotic cells and many bacteria. Apart from this vital function, coenzymes Q_n act as effective antioxidants protecting cells from damage by free radicals, such as structurally similar vitamin E. In mammals, the most common form is coenzyme Q_{10} (also known as ubiquinone-10 or ubidecarenone), which contains ten isoprene units. Coenzyme Q_{10} co-exists with its reduced form $CoQ_{10}H_2$, known as ubiquinol-10. Coenzyme Q_{10} is derived from 4-hydroxybenzoic acid (*p*-hydroxybenzoic acid), though the origin of this compound varies according to the organisms. Thus, bacteria are known to produce coenzyme Q_{10} from chorismic acid, whereas plants and animals utilise a route from L-phenylalanine or L-tyrosine via 4-hydroxycinnamic acid (4-coumaric acid). The adult human body pool of coenzyme Q_{10} has been found to be approximately 2 g and requires replacement of about 500 mg per day. This must be supplied either by endogenous synthesis or from exogenous sources. Reduced biosynthesis and increased utilisation by the body (increasingly with age) are the major factors that lead to deficiency of coenzyme Q_{10} in humans. Coenzyme Q_{10} is found in almost all foods of animal and vegetable origin. The average daily content of the western diet is 3–5 mg. It is met mostly by meat, poultry and fish and to some extent by food of vegetable origin. Higher amounts of coenzyme Q_{10} are found in meat (14–54 mg/kg), liver (26–50 mg/kg), fish (4–67 mg/kg), vegetable oils (4–280 mg/kg) and nuts (2–27 mg/kg). Supplementation appears to be the way for older people, and certainly the ill, to provide the major proportion of the 500 mg/day needed, however the optimal intake is not yet known, as the available clinical trials have provided conflicting results.



5-137, coenzymes Q (ubiquinones), $n = 1–12$



5-138, plastoquinones, $n = 3–10$

In plants, coenzymes Q_n are accompanied by other lipophilic compounds of similar structure, plastoquinones (**5-138**), vitamin E and vitamin K_1. Similarly to tocopherols and tocotrienols, plastoquinones are produced from homogentisic acid by *C*-alkylation (*ortho* to the OH group) using polyisoprenyl diphosphate with $n = 3–10$, but most commonly with $n = 9$, which is called solanesyl diphosphate. Plastoquinones are involved in the photosynthetic electron transport chain in plants.

6

Minerals

6.1 Introduction

The chemical composition of food can be viewed either in terms of material composition, which is the representation of individual compounds, or in terms of elemental composition (content of individual elements). If we leave aside water, the bulk of the materials are organic food substances. The main constitutional elements of the organic compounds are non-metals: carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P) and sulfur (S). These elements are called **organogenic elements**. Other chemical elements contained in food are **minerals**. The elements phosphorus and sulfur belong to both groups.

Minerals in food (dietary minerals, also known as mineral nutrients) are the chemical elements required by living organisms (other than carbon, hydrogen, nitrogen and oxygen present in common organic molecules) that are usually defined as the elements contained in the ash of a food, or more accurately as the elements that remain after the complete oxidation of the organic fraction of food to carbon dioxide, water and other oxidation products. The mineral fraction for most foods accounts for 0.5–3% by weight.

Minerals can be classified according to various criteria, for example with regard to concentration, biological and nutritional importance, dietetic effects and origin. According to the amount present, minerals are divided into the following four groups:

- **Majority mineral elements** Or quantity elements, formerly referred to as **macroelements**, which occur in food in larger amounts, usually in hundredths to units of weight per cent (hundreds to ten thousands mg/kg) and include alkali metals sodium (Na) and potassium (K), alkaline earth metals magnesium (Mg) and calcium (Ca), halogen chlorine (Cl) and non-metals phosphorus (P) and sulfur (S).

- **Minority mineral elements** These are found in foods in smaller amounts, representing the tens to hundreds mg/kg; a transition between the majority elements and trace elements; this category usually includes transition metals iron (Fe) and zinc (Zn).

- **Trace elements** or **microelements** Occurring in even lower concentrations (tens of mg/kg or less); important trace elements are transition metals cobalt (Co), chromium (Cr), copper (Cu), Fe (sometimes), manganese (Mn), molybdenum (Mo), nickel (Ni) and sometimes zinc (Zn), post-transition metal aluminium (Al), the halogen iodine (I) and non-metal selenium (Se).

- **Ultra trace elements** Normally comprising less than 1 μg/g (less than 0.0001% by weight); possible ultra trace elements in humans include the metalloids boron (B) and silicon (Si), the halogen fluorine (F), metalloid arsenic (As) and the transition metal vanadium (V); other possible ultra trace elements in other organisms include the alkali metal lithium (Li), halogens fluorine (F) and bromine (Br), transition metals cadmium (Cd) and tungsten, also known as wolfram, (W) and post-transition metals lead (Pb) and tin (Sn). Despite demonstrations of their roles in experimental animals, the exact function of these elements in human tissues and their importance for human health are uncertain.

The classification of minerals in foods to majority, minority, trace and ultra trace elements roughly corresponds to the occurrence of these elements in the human organism (Table 6.1).

The mineral contents in food are very different and extremely variable within the individual commodities and even within one commodity. This is due to differences in the metabolism of elements of diverse organisms, genetic factors and, in particular, conditions of production of the raw materials. Minerals originate in the soil and cannot be created by living organisms. Plants absorb minerals from the soil and animals obtain their minerals from the plants or other animals that they eat. Most of the minerals in the human diet come directly from foods of plant origin or indirectly from animal sources. Minerals from plant sources may vary from place to place, because the mineral content of the soil varies according to the location in which the plant was grown, and it further depends on soil characteristics, mode and degree of fertilisation, climatic conditions, level of maturity of crops and other factors. The mineral

The Chemistry of Food, First Edition. Jan Velíšek.
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Table 6.1 Minerals content in the body of an adult (body weight 70 kg).

Element	Total amount	Unit	Element	Total amount	Unit
Ca	1000–1500	g	Si	1.4	g
Mg	25–40	g	Cu	100–180	mg
K	140–180	g	Mn	10–20	mg
Na	70–100	g	Mo	5–10	mg
P	420–840	g	Co	1–1.5	mg
S	cca 140	g	Ni	10	mg
Cl	70–110	g	Cr	5	mg
Fe	3–5	g	V	<1–20	mg
Zn	1.4–3	g	I	10–30	mg
F	0.8–2.5	g	Se	10–20	mg

content of foods of animal origin depends on nutrition, age and state of health.

The general classification of elements as majority, minority, trace or ultra trace elements is therefore only approximate. For example, Al is present in various spices and tea in hundreds of milligrams per kilogram, so from this perspective it is one of the minor elements, while in milk it may be classified as a trace element or ultra trace element (because it is usually found in concentrations of less than 0.1 mg/kg). Another example of the difficulty in classification of elements is Mn. In animal products, such as beef or pork flesh, Mn is found only in traces (0.1–0.2 mg/kg), but about a 100-fold to 300-fold higher amount is found in cereals (wheat 35–49, rye 31–44, barley 16–25 and oats 45–72 mg/kg) and approximately 1000-fold higher level (300–1000 mg/kg) is present in tea leaves. In some food materials that are highly purified (refined sugar, refined oils), the total content of minerals is very low (up to hundredths to tenths of a per cent), so within these foods the common majority elements (Ca, P, K) are trace elements.

According to their physiological significance, minerals in foods are divided into three groups, namely:

- **Essential elements** Also known as **necessary** (**obligatory**) elements that the body must receive from food in certain amounts to ensure important biological functions (e.g. construction of biological structures, catalytic, regulatory and protective functions); the essential elements are all the majority elements (Na, K, Mg, Ca, Cl, P and S) and many trace elements (such as Fe, Zn, Mn, Cu, Ni, Co, Mo, Cr, Se, I, F, B and Si).

- **Non-essential elements** Physiologically indifferent elements or elements with as yet unknown biological functions that are not significantly toxic; this group includes all other chemical elements in food that usually occur in trace amounts (e.g. Li, Rb, Cs, Ti, Au, Sn, Bi, Te and Br); these elements sometimes regularly accompany essential elements (e.g. Li accompanies Na, and Rb accompanies K).

- **Toxic elements** Elements that have no biological role and are poisonous in the elemental form or produce poisonous soluble compounds exhibiting toxic effects; mechanisms of these effects often lie in the inhibition of metabolically significant enzymes as a consequence of interaction of the toxic element with the enzyme molecule; the most important toxic elements in food are particular metals (Pb, Cd and Hg) and metalloids (As).

Classification of an element into these groups is not definitive and depends on the specific biological species for which the element is essential. Furthermore, the toxic effects of certain metals are different for different organisms. It should be noted that even the essential elements (e.g. Se and Ni) can be toxic at higher doses. On the other hand, As, whose toxic effects have been known for a long time, is a physiological stimulating factor for some animals.

A certain element may be included among the essential elements of a larger group of animals if it satisfies the following conditions:

- the element is present in all healthy tissues of the body;
- its concentrations in the same body tissues of different species are similar;
- its exclusion from the diet leads to repeated physiological abnormalities;
- re-introduction to the deficient diet results in normal physiological state;
- complete and long-term elimination of the element from the diet results in the death of the organism.

Elements that do not meet all these criteria, even if there is evidence of positive effects of their presence in a nutritionally balanced diet, are not essential. They are referred to as **functionally beneficial**. Some elements can be found in characteristic quantities in the bodies of all organisms (such as alkali elements, P, S, Cl, Fe, Zn, Cu and Mn) and these are termed **invariable elements**. Other elements, known as **variable elements**, occur in higher concentrations only in some organisms. For example, V is present in blood cells of some tunicates, also known as urochordates or in fruiting bodies of some fungi (see Section 6.3.14.2), while in most other organisms it belongs to the trace or ultra trace elements.

Most of the elements discussed so far are natural food components. This means that they are present in a given food commodity in a characteristic amount, which is a consequence of a cycle of elements in nature and their natural distribution in different parts of the biosphere.

The presence in food of a higher amount of an element may be the result of contamination during the manufacturing process, or of the raw material during the agricultural production. This element is then considered a contaminant (e.g. toxic elements Pb, Hg, Cd, As, Tl and Sb). Any essential element (e.g. Cu, Co, Cr, Fe, Mn, Ni, Se and Zn) may also become a contaminant if its content

in food is significantly higher than the typical concentrations. Some anions also have toxic effects (see Section 6.6), as do some radionuclides (see Section 6.7).

For selected elements, the Recommended Daily Allowance (RDA) are set in the EU (first number) and United States (second number) as follows: K (2000/4700 mg), Cl (800/2300 mg), Ca (800/1000 mg), P (700/700 mg), Fe (14/8 mg), mg (375/400 mg), Zn (10/11 mg), Cu (1/0.9 mg), Mn (2/2.3 mg), F (3.5/4 mg), Se (55/55 μg), Cr (40/35 μg), Mo (50/45 μg) and I (150/150 μg). Insufficient intake of some essential elements through food can be solved by enrichment. For example, Ca can be added to dairy milk at a level of 30%, and I at 20% of the recommended daily allowances. Generally, food enrichment with calcium is through different calcium salts (carbonate, chloride, citrate, gluconate, lactate and phosphate), calcium oxide and hydroxide; iodine is added in the form of sodium or potassium iodide. If the element enrichment only compensates for the losses that occur during technological processing of raw materials, we talk about **restitution**. If the level of the element is increased above its natural concentration in the food by enrichment, it is called **fortification**. In both cases, the element is considered a food additive. Dietary supplements (also known as food supplements or nutritional supplements) may contain a single element, a mixture of different dietary minerals or combination of minerals together with vitamins and/or other beneficial compounds.

This chapter is devoted to mineral substances in foods, and deals first with their chemistry, binding options and interactions with food ingredients. All of the majority, minority, trace, ultra trace and non-essential mineral elements are described, and their presence in food, biochemical and physiological functions, metabolism, nutritional significance and health consequences are discussed. The next part of the chapter deals with toxic elements and toxic anionts, their occurrence in foods and the environment, dietary intake, metabolism, toxic effects and toxicological evaluation. The final part, dealing with radionuclides and radioactivity, concentrates on radioactive nuclides occurring in the environment and foods and their health implications.

6.2 Chemistry of minerals

Mineral substances found in food interact with water, with the organic matter present and with one another. These interactions affect the bioavailability of elements in the diet. The chemical state of the element in the food is determined by food composition, pH, the possibility of hydration of the metal ions, redox potential of the system and the related possibility of changing the degree of oxidation of the element and other factors. The solubility of the individual substances is closely associated with the resorption properties of the minerals. Food contains a number of components, such as amino acids, peptides, proteins, carbohydrates, lignin, phytic acid, organic acids and other compounds, which can bind minerals and thereby influence their biological availability. Interactions of various elements with organic molecules are studied in bioinorganic chemistry.

6.2.1 Bonding possibilities of elements

The chemical properties of the given element (depending on the form of the products) are crucial in the interactions of the element in the organic food matrix. These properties are determined by the position of the element in the Periodic Table and are a result of the electron configuration in the atom of the element.

Non-metals and metalloids with medium electronegativity values[1] (P, As, S and Se) form **covalent compounds** in biological systems, such as esters of phosphoric, pyrophosphoric and tripolyphosphoric acids, sulfur amino acids and their selenium analogues, arsenic analogues of amino compounds and sulfur heterocycles.

Elements with very low electronegativity (alkali metals, alkaline earth metals, such as Na, Ca and Mg) and elements with high electronegativity (halogens such as Cl and I) occur mainly as free ions in biological materials, and are preferably involved in electrostatic interactions. However, even these elements can form less soluble compounds (calcium oxalate), covalent compounds (hormones thyroxine and triiodothyronine are iodinated aromatic amino acids, see Section 2.2.1.2.5) or complex compounds (chlorides as ligands and some metal ions as central atoms). A ligand is an entity (atom, ion or molecule), which can act as an electron pair acceptor to create a coordinate covalent bond with the central ion. Cd and Hg also tend to form covalent compounds.

Transition metals and some post-transition metals (Al, Pb and Zn) have a strong tendency to form complex compounds. This is due, among other things, to the smaller radius and larger electric charge of their ions. These elements have a large number of potential reaction partners (ligands) in biological materials. The stability of complexes depends on the type of ligand donor atoms, the metal and its oxidation state together with steric factors. Divalent cations of the first transitional series, for example biologically important Cu^{2+} ions, form the most stable complexes. The preferred ligands of Cu^{2+} ions are ligands containing sulfur and nitrogen or several nitrogen atoms as donors. Conversely, Mn^{2+} ions form complexes fairly reluctantly, and in addition to ligands with sulfur and nitrogen entities with oxygen donor atoms can also act as ligands (Figure 6.1). Metal ions in oxidation

[1] Electronegativity is the ability of the covalently bound atom of an element to attract electrons. The light halogens and oxygen have the highest electronegativity and the heavier alkali metals have the lowest electronegativity. In general, the metals have lower electonegativities than the non-metals. Hydrogen has a medium electronegativity (2.1 according to the Pauling scale) and represents the transition between the two groups (of the metals, only Au and Pt have a higher electronegativity than hydrogen). For selected elements, the order of decreasing electronegativity is as follows: $F \gg O \gg Cl > N > Br > S = I > C = Se > P = H > As = B > Hg = Cu = Ag = Sb > Si = Sn = Pb = Tl = Fe = Co = Ni = Mo > Cd > Zn = Cr = V > Al = Mn = Ti = Be > Sc > Y > La = Ac > Mg > Ca = Sr = Li > Na = Ba > K = Rb > Cs = Fr$.

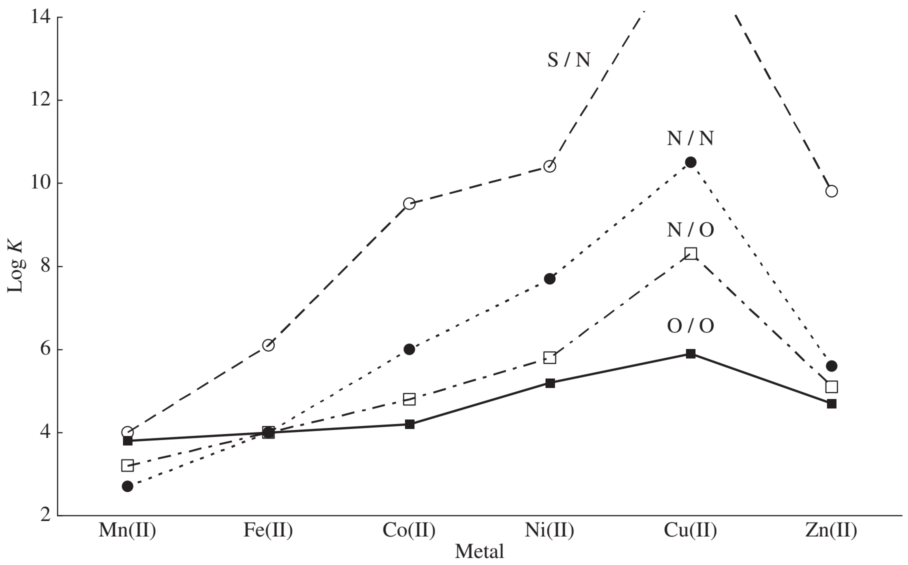


Figure 6.1 Dependence of the logarithm of stability constants (log *K*) of divalent metal cations complexes of the first transitional series on the type of ligand according to the type of donor atoms: (── oxalic acid-oxygen/oxygen; ·-·-·-· glycine-nitrogen/oxygen; ······· ethylenediamine-nitrogen/nitrogen; ------cysteine-sulfur/nitrogen). da Silva and Williams, 2001, fig 2.8. Reproduced by permission of Oxford University Press.

states III and IV are generally more stable than the corresponding cationic complexes in oxidation state II. Metal cations with higher charge may, however, in the neutral or weakly acidic environments typical of foods, form insoluble hydroxides, for example $Fe(OH)_3$, oxides (MnO_2) or oxocations (TiO^{2+}, VO^{2+} and BiO^{+}). Some metal ions in a middle oxidation state, unless they are bound in complexes, are susceptible to disproportionation (e.g. $2\ Mn^{3+} + 2\ H_2O \rightarrow Mn^{2+} + MnO_2 + 4\ H^{+}$). With the exception of Mo, which is commonly in the very stable oxidation state VI, transition metals do not occur in high oxidation states in biological materials (e.g. oxoanions MnO_4^{-}, MnO_4^{2-} and CrO_4^{2-}). Various forms of minerals in foods are summarised in Table 6.2.

6.2.2 Interaction with organic food components

6.2.2.1 Aminocarboxylic acids

Aminocarboxylic acids can bind metal ions as coordination compounds via dissociated carboxyl groups and amino groups. The donor atoms are the oxygen atom in the carboxyl group and

Table 6.2 Overview of forms of minerals in foods.

Form	Example
Elemental form	Fe in some fortified foods
Free and hydrated ions of metals and non-metals in different oxidation states	Cu^{2+}, $Cu(H_2O)_4^{2+}$, $Fe(H_2O)_6^{2+}$, $Fe(H_2O)_6^{3+}$, AsO_3^{3-}, AsO_4^{3-}
Complex compounds of metals with inorganic ligands	$CuCl_4^{2-}$, $Cu(NH_3)_4^{2+}$
Complex compounds of metals with organic ligands	Compounds with amino acids, peptides, proteins, carbohydrates, phytic acid, hydroxycarboxylic acids, plant phenols (flavonoids) and porphyrins
Minerals bound to insoluble biopolymers	Binding to various components of fiber
Slightly soluble compounds	Sulfides, sulfates, phosphates, oxalates, hydroxides, phytates
Covalent compounds of non-metallic and semimetallic elements	Phytic acid, sulfur amino acids and their selenium analogues, arsenic analogues of amino compounds
Organometallic compounds	Methylmercury, dimethylmercury, tetraethyllead

the nitrogen atom in amino group, which are able to provide an electron pair to the coordinate covalent bonds with the central metal ion. The stability of complexes of bivalent metal cations with amino acids decreases according to the bound metal in the order:

$$Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Cd^{2+} > Fe^{2+} > Mn^{2+}$$

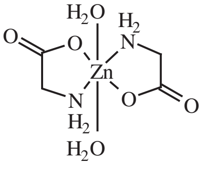
For a given metal, however, the stability constants of complexes with different amino acids can differ by more than two orders of magnitude. Amino acids and derived compounds (peptides and proteins) have amphoteric character and occur in an aqueous medium, depending on the pH, in various forms, which differ in their dissociations of the functional groups. The presences of these groups in the ligand molecule and the pH value have a decisive influence on the formation of complexes with metal ions. The participation of various functional groups in metal binding depends on two conditions:

- how the relevant functional group competes with other groups in the vicinity of the metal ions;
- how metal ions compete with hydrogen ions for the binding sites in the ligand functional groups.

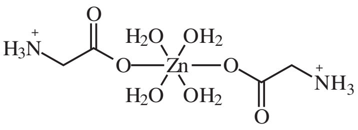
Dissociation constant values of various functional groups of amino acids thus affect the complex formation. The lower the pK value, the greater the ability of the ligand donor atom to bind the metal. In a certain pH range, more functional groups can act as electron acceptors, which lead to the formation of cyclic complexes, **chelates**, which are thermodynamically more stable than complexes with monofunctional ligands. The ability to form a chelate of suitable coordination geometry depends on whether the ligand molecule configuration allows the creation of five- to seven-membered rings. Amino groups of amino acids are, despite of high pK values, often involved in coordination bonds with metals together with the amino acid anion. The nucleophilic character of the nitrogen atom facilitates coordination.

The effect of the acid–base equilibrium on the structure of amino acid–metal complexes can be demonstrated by glycine complexes with zinc. In neutral media, both functional groups of glycine (Gly) are involved in the binding of zinc, yielding the chelate $Zn(Gly)_2{\cdot}2H_2O$ (**6-1**). In acidic media, amino groups are protonised, so the metal is bound only by the carboxyl groups and the tetrahydrate of the zinc salt of glycine, $Zn(Gly)_2.4H_2O$ (**6-2**), forms. The nucleophilic character of nitrogen facilitates the coordination.

Other functional groups of amino acids may also contribute to the formation of complexes with metals and stabilise them through

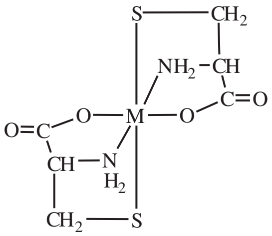


6-1, diaqua-bis(glycinato)zinc complex



6-2, glycine zinc salt tetrahydrate

additional bonds. Metals and metalloids with a distinctive affinity for sulfur (such as Hg, Cd, Ag, Cu, Bi, Sb and As) yield very stable compounds with thiols. The participation of the sulfhydryl group in cysteine in bonds with metal ions leads to a complex of higher stability (**6-3**). Stability constants of cysteine complexes with Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} a Pb^{2+} are significantly higher than the corresponding glycine and histidine complexes. Unlike sulfhydryl groups, hydroxyl groups in serine, threonine and tyrosine do not contribute significantly in the metal binding.

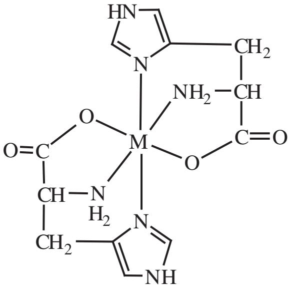


6-3, complex of metal (M) with cysteine (M = Cu, Zn, Cd, Mn, Ni, Fe, Pb)

Often, however, the imidazole nucleus of histidine (free and bound histidine in peptides and proteins) is involved in metal binding. The order of dissociation of functional groups in the molecule of histidine is evident from the following pK values: pK_1 (COOH) = 1.80, pK_2 (imidazolium) = 6.04, pK_3 (NH_3^+) = 9.33, pK_4 (imidazole) = 14. This results in the following stages during the formation of histidine complexes with metals:

- coordination at the oxygen atom of the carboxyl, even at low pH value
- metal binding to the nitrogen atom of the imidazole nucleus
- metal binding to the amino group nitrogen.

All three types of bonds occur in complexes such as bis-L-histidinatonickel or bis-L-histidinatocobalt (**6-4**). Stable



6-4, complex of metal (M) with histidine (M = Co, Ni)

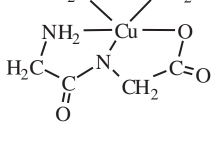
complexes also form amines and diamines (products of decarboxylation of amino acids) such as histamine, cysteamine, putrescine and cadaverine (see Section 10.3.2.10).

As well as the common basic amino acids and their homologues, such as ornithine and homocysteine, some less common amino acids may also act as metal ligands. Examples are heterocyclic amino acids with an azetidine ring (see Section 2.2.1.2.10), especially nicotianamine (2-71) and its hydroxylated derivative mugineic acid (2-72). Amino acids of this type are, among other substances, excreted by plants in the vicinity of the roots, and the complexing action solubilises iron and some other elements in the soil. The metals are then more accessible to the root system of the plants. In relation to this function, these compounds are referred to as **phytosiderophores**. Nicotianamine was found in some plants (called heavy metal hyperacumulators) to have an extraordinary ability to accumulate particular metals from the soil, especially nickel and zinc. However, other than nicotianamine, further ligands, especially hydroxycarboxylic acids, are similarly involved in the binding of metals accumulated in excessive amounts in plant tissues. Another important natural ligand, ethylenediaminetetraacetic acid (EDTA), was recently found in some hyperaccumulating herbs. This compound has been widely used in analytical chemistry (where it is known as Chelatone 3), in chemical technology and as a food additive (see Section 11.3.3).

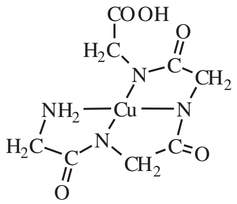
6.2.2.2 Peptides and proteins

Peptides and proteins can bind metals through the *N*-terminal amino group, *C*-terminal carboxyl and functional groups in the side chain of amino acid residues. This is particularly true of amino acids such as lysine (Lys), ornithine (Orn), aspartic acid (Asp), glutamic acid (Glu), cysteine (Cys) and histidine (His). Another option is to bind the metal to the oxygen or nitrogen atom of the peptide bond. The oxygen atom of the carbonyl group of the peptide bond has only a slight nucleophilic character, so that metal binding is weak. The neighbouring amino group can provide chelate stabilisation. Binding of metal ions on the nitrogen atom of the peptide bond assumes that dissociation of the NH group as an electron pair on the nitrogen is not available.

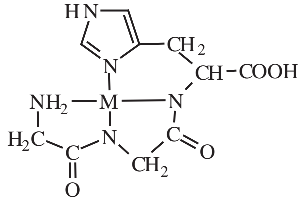
In metal complexes of some oligopeptides, such as Gly-Gly, Gly-Gly-Gly-Gly or Gly-Gly-His, a peptide chain surrounds the central metal ion and creates bonds with the participation of all sterically accessible donor groups. Alternatively, free coordination sites are occupied by water molecules (**6-5** to **6-7**). In some simple peptides, such as Gly-Gly, the structure of their complexes with metals may be rather complex and multinucleated complexes may arise, such as $Zn_2(Gly\text{-}Gly)_4.2H_2O$.



6-5, complex of copper with dipeptide Gly-Gly



6-6, complex of copper with tetrapeptide Gly-Gly-Gly-Gly



6-7, complex of metal (M) with tripeptide Gly-Gly-His

Metallothioneins, a family of cysteine-rich metal-binding peptides and low molecular weight proteins (molecular weight of 3.5–14 kDa), occupy a special role among naturally occurring compounds that act as metal ligands. Metallothioneins are found in the bodies of many microorganisms, plants, fungi, invertebrates and vertebrates, including humans. They were originally found in the internal organs of mammals, such as the kidneys, liver, intestines, spleen and pancreas of horses, cattle and other livestock. Metallothioneins ensure detoxification of toxic metals (such as Cd) by formation of stable complexes. Stable complexes with essential metals (e.g. Zn and Cu) are employed for temporary storage of metals in tissues until they are required for the synthesis of metalloproteins or other substances. They also provide protection against oxidative stress. The first categorisation of metallothioneins (MTs) established three classes: class I, includes the metallothioneins that are homologous with horse metallothionein; class II, includes the rest of the metallothioneins with no homology with horse metallothionein; and class III, which includes phytochelatins.[2] All mammals express at least the main isoforms of class I metallothioneins, designated as MT1, MT2, MT3 and MT4. MT1 and MT2 are expressed in almost all tissues, whereas MT3 and MT4 are tissue-specific.

Metallothioneins of class I are thermostable and acid resistant peptides characterised by a molecular weight of 6–7 kDa, containing 60–68 amino acid residues, including 20 cysteine residues (cysteine residues represent about 30% of the amino acid content of metallothioneins) and binding a total of seven equivalents of bivalent metal ions. Aromatic amino acids are usually absent. The positions of the cysteine and lysine units in peptide chains are almost identical in various types of mammalian metallothioneins. The *N*-terminal amino acid is *N*-acetylmethionine (Figure 6.2).

[2]The current classification divides metallothioneins into 15 groups and is based on taxonomic parameters and the pattern of distribution of cysteine residues along the metallothionein sequence. Family 15 contains the plant metallothioneins later classified into four different types depending on the distribution of their cysteine residues and cysteine-devoid regions.

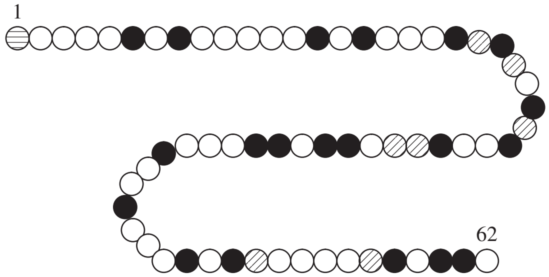
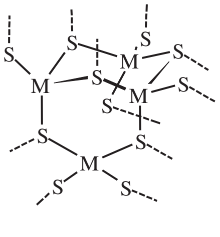


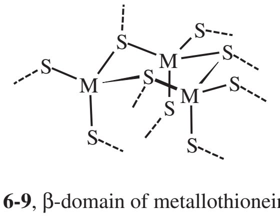
Figure 6.2 Cysteine (full circles), lysine (obliquely shaded circles) and *N*-acetylmethionine (horizontally hatched circle) positions in the sequence of amino acids in mammalian metallothioneine.

All cysteine residues occur in the reduced form and are coordinated to the metal ions through mercaptide bonds. Despite these common structural features, metallothioneins are organ-specific species. Their increased synthesis in the body is induced by higher dietary intake of metals (Cd, Cu, Zn, Ni, Pb, Co, Bi, Hg, Au and Ag) or exposure in the environment in which an organism lives. The synthesis of metallothioneins is most strongly induced by Cd. One peptide molecule can bind up to seven atoms of divalent metals. Complexes isolated from animal tissues almost always contain Zn and Cd. These substances, however, still have binding capacity, which allows the fixing of the other metal ions. Detailed studies of the metallothionein structure have found that the metal atoms are bound in two domains, α and β. Domain α (**6-8**) is located near the *C*-terminus of the peptide chain and the participation of 11 sulfur atoms fixes the four metal atoms. Domain β (**6-9**) is located near the *N*-terminus of the peptide chain and the participation of nine sulfur atoms fixes three metal atoms (M). Cu in the monovalent form may be bound in complexes with metallothionein in even larger quantities (12 Cu^{I} atoms in one molecule). Cu–thioneins are characterised by a distinctive orange to red fluorescence when excited by UV radiation.

Another type of class I metallothionein, known as MT3, contains bound Cu and Zn and occurs in brain tissue. A typical representative is composed of 68 amino acids and is similar to metallothioneins of crustaceans, which contain 18 cysteine residues. Similar binding peptides were also found in other animals exposed to toxic elements, for example in fish and invertebrates.



6-8, α-domain of metallothioneine (M = metal)



6-9, β-domain of metallothioneine (M = metal)

Metallothioneins of class II also contain a high proportion of cysteine units and their positions in various polypeptides of this group are quite different. A molecule of these peptides contains the characteristic repeating units Cys-Cys and Cys-X-Cys or Cys-X-X-Cys, where X is an amino acid other than cysteine. These polypeptides were found in several economically important crops such as wheat, maize, rice, buckwheat and cotton, but often only in certain parts of the plant and at specific stages of its growth.

Class III metallothioneins represent plant and microbial peptides called **phytochelatins** (previously known as cadystins or EC-peptides). These peptides are oligomers of glutathione, produced by the enzyme phytochelatin synthase, and have a primary structure $(\gamma\text{Glu-Cys})_n$-Gly, where $n = 2$–11. Phytochelatins also includes homophytochelatins, $(\gamma\text{Glu-Cys})_n$-β-Ala, homoglutathione oligomers, (glycine is replaced by β-alanine), hydroxymethylphytochelatins (hydroxyglutathione oligomers), $(\gamma\text{Glu-Cys})_n$-Ser, with serine as the *C*-terminal amino acid and isophytochelatins that have their amino acid sequence terminated by glutamic acid (glutamylcysteinylglutamate derivatives) or glutamine, $(\gamma\text{Glu-Cys})_n$-Glx. Other groups include deglycylphytochelatins, $(\gamma\text{Glu-Cys})_n$ and deglutamylphytochelatins with the structure Cys-$(\gamma\text{Glu-Cys})_n$-Gly. Phytochelatins and related substances in the cells are able to bind heavy metal ions into stable complexes, which are then stored in the vacuoles. Phytochelatins can be found in yeasts, algae, mosses and even higher plants. Their formation in plant tissues is probably to protect the plant against the toxic effects of heavy metals. They also occur in some agriculturally important plants, such as potatoes, beans, wheat, barley or maize. Legume plants (family Fabaceae) primarily contain homophytochelatins, while grasses such as cereals (members of the monocot family Poaceae), with the exception of maize, synthesise hydroxymethylphytochelatins.

Binding of metals in biological materials is largely mediated by proteins. This binding of the metal ions by the protein molecule proceeds by a similar mechanisms to that for the amino acids and peptides. The functional groups of proteins that are involved in metal binding are not necessarily the amino acid residues that are adjacent in the polypeptide chains. The close contact with the metal ion due to the tertiary structure of the proteins can also extend to those parts of the macromolecule that are fairly distant from one another in the amino acid sequence. Another possibility is the binding of metals by interaction with phosphate groups of phosphoproteins (e.g. glycophosphoprotein of egg yolk, called phosvitin, forms complexes through the serine residues with Fe^{3+}, Ca^{2+} or Mg^{2+}). The possibility of interactions of proteins with metal ions and the stability of the resulting complexes depends on temperature, pH, the type of metal ion, the presence of other components in the primary structure and protein conformation.

Metal complexes of proteins that occur in foods can be of two types. The first group of complexes, arising randomly, is relatively labile. Owing to the large number of reactive functional groups, practically every protein interacts with metal ions under appropriate

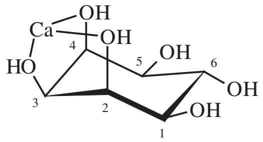
conditions (pH value). The constitution of such a complex is not uniform and demonstrates the statistical distribution of the individual particles, which reflects the degree of dissociation of the functional groups of the protein. The stability and properties of such complexes can only be partially estimated on the basis of theory, so in each particular case it is necessary to examine them experimentally. Higher concentrations of some metal ions can lead to protein denaturation.

The second group of metal complexes with proteins are **metalloproteins**, which have a regular structure, metal binding sites for the metal ion and a characteristic way of binding. The metal can also be bound by substances other than amino acids in the protein macromolecule, for example prosthetic groups (Fe bound in the porphyrin structure of haem in haemoglobin, myoglobin and haem enzymes). Some metalloproteins have very important biological functions; metalloproteins act as catalysts (metalloenzymes), transport and storage compounds (Table 6.3). A number of important metalloenzymes are mentioned in the relevant sections dealing with the individual elements.

6.2.2.3 Saccharides and their derivatives

In principle, polyhydroxy compounds such as saccharides and sugar alcohols also have chelating effects (due to the affinity of the chelating ligands for a metal ion). These compounds, however, (with the exception of the special steric arrangement) occur only under exceptional chemical conditions, which foods do not usually encounter. The oxygen atom of the hydroxyl group is for most metals a much weaker donor of an electron pair than atoms of nitrogen or sulfur. Monosaccharides, disaccharides and sugar alcohols form complexes with metal ions in an alkaline medium. Complexes with ferric ions are coloured yellow to dark brown, and the stoichiometric ratio of Fe and sugar is 1 : 1. The study of these compounds was initiated because of the finding that the addition of some sugars in the diet (especially D-fructose and D-glucitol) increases the bioavailability of iron. Aldoses and ketoses may form complexes with metals in acyclic (hydrates) or cyclic forms (furanoses and pyranoses). For example, in acidic media, D-altrose (also D-arabinose and D-idose) predominantly form complexes in their acyclic forms with hexavalent Mo ions. In the binding of ions, the hydroxyl group of the hydrated carbonyl group and the secondary hydroxyl group at C-2, C-3 and C-4 act as donors. The aldopyranoses D-allose and D-gulose form complexes under the participation of hydroxyls at C-2, C-3, C-4 and C-6. Similar complexes are formed from pentose D-ribose. Derivatives of carbohydrates, such as aldonic and alduronic acids, 2-amino-2-deoxysugars and sugar phosphates, have an even stronger tendency to form complexes.

Spontaneous formation of complexes of carbohydrates and related compounds with metal ions in food can be expected for those substances where configuration of the hydroxyl groups on the three adjacent carbon atoms in the six-membered rings of the chair conformations are alternately axial, equatorial and axial. This is the case for the *epi*-inositol–Ca^{2+} complex (**6-10**).



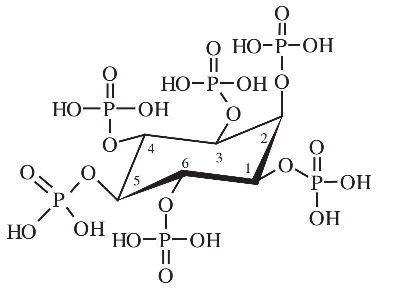
6-10, complex of calcium with *epi*-inositol

Phosphorylated organic substrates (e.g. sugar phosphates) are weak acids and can form salts with various metals. An important binding agent for metallic elements is **phytic acid** (*myo*-inositol hexakisdihydrogen phosphate). Conformation of phytic acid in solutions of pH 0.5–9 (one phosphate group in the axial position and five phosphate groups in equatorial positions) is represented by the formula shown in **6-11**. In solutions of pH > 9.5, five phosphate groups are bound in the axial positions and one in

Table 6.3 Some important metalloproteins.

Element	Metalloprotein	Occurrence	Element	Metalloprotein	Occurrence
Ca	Calmodulin	Muscle	Cu	Plantacyanin	Spinach
	Parvalbumin	Muscle		Ceruloplasmin	Blood plasma
	Troponin C	Muscle		Cerebrocuprein	Brain
Fe	Myoglobin	Muscle		Haemocuprein	Blood
	Haemoglobin	Erythrocytes	Mo	Xanthinoxidase	Liver
	Cytochromes, catalases, peroxidases	Widespread	Fe (Cu, Zn, Mn)	Conalbumin	Egg white
	Transferrin	Blood, liver	Ni	Nickelplasmin	Krevní plasma
	Ferritin	Spleen		Urease	Soy, rice
	Lactoferrin	Milk	Mn	Pyruvate decarboxylase	Widespread
	Ferredoxins	Spinach		Arginase	Liver

the equatorial position. At pH 9.5, both chair conformations are present in equimolar concentrations.



6-11, phytic acid

Phytic acid forms stable complexes with calcium, magnesium, iron, zinc and other metal ions, so-called **phytates**, in ratios of 1 : 1 to 1 : 6. The calcium–magnesium complex of phytic acid is known as **phytin** (or calcium–magnesium phytate). Binding of various metals on phytic acid at pH = 7.4 drops in the order: Cu > Zn > Ni > Co > Mn > Fe > Ca. The strong binding of the elements in these compounds and low solubility (iron phytate) results in decreased bioavailability of the bound elements in diets that contain higher amounts of phytic acid and phytin, which may, however, prevent colon cancer by reducing oxidative stress in the lumen of the intestinal tract. Phytic acid and its salts with metals are found in foods of plant origin, especially cereals, legumes, oil seeds and nut hulls (see Section 6.3.4.2.1).

Owing to the negative charges of the dissociated phosphate groups, phytic acid (even with bound metal) can react with ionised proteins or with some phospholipids (Figure 6.3), and the complexes phytic acid–protein, metal–phytic acid–protein or phytic acid–metal–phospholipid are formed. In an acid medium, the proteins are bound to phytic acid by positively charged amino groups of the basic amino acids (Lys, Arg and His) and by the terminal amino groups. At a pH value equal to the pH of the protein isoelectric point, only weak interactions proceed. In neutral and alkaline media, phytate complexes with metals bind to proteins through ionised carboxyl groups. Crude soybean oil, for example, contains approximately 50–340 mg/kg phytate, which is largely removed during oil degumming and passes, together with the phospholipid fraction, into the lecithin fraction. Degummed oil contains phytates at a level of about 4–50 mg/kg.

During the sugar juice clarification (known as carbonation; see Section 4.4.2.1.2) using calcium hydroxide, saccharose dissociates in an alkaline medium in several steps (at 25 °C: $pK_1 = 12.60$; $pK_2 = 13.52$; $pK_3 = 13.72$; $pK_4 = 13.77$). The most reactive atoms are hydrogens of the primary hydroxyl at C-6 of the glucose moiety, and C-1 and C-6 of fructose moiety. It is reported that in solutions of pH 11, about 10% of sucrose is present in the form of

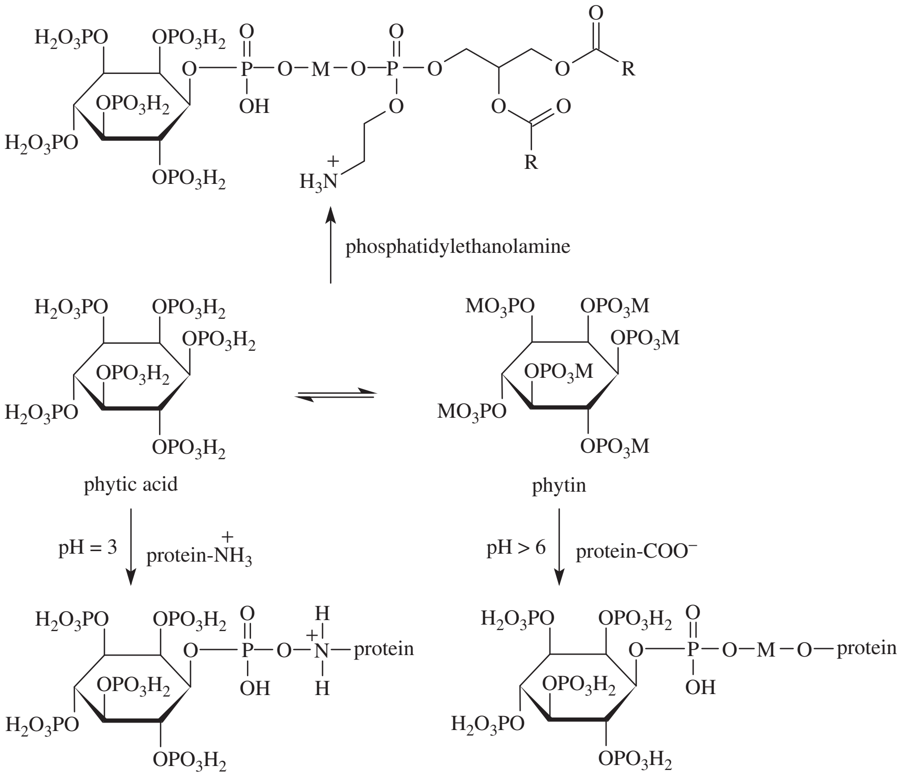


Figure 6.3 Reactions of phytic acid and phytin with metal ions (M = Ca, Mg, Zn, Fe, Cu, Mn), proteins and phospholipids (R = fatty acid residue).

monovalent anions, at pH 12.2 about 50% of the molecules, and at pH 12.5 bivalent anions[3] are also present. Depending on the amount of added lime milk (pH of the juice) and the temperature, initially soluble monocalcium saccharate (with the molecular formula $C_{12}H_{22}O_{11}{\cdot}CaO$) and slightly soluble dicalcium saccharate ($C_{12}H_{22}O_{11}{\cdot}2CaO$) form, followed at higher pH and higher temperatures by insoluble tricalcium saccharate ($C_{12}H_{22}O_{11}{\cdot}3CaO$). In solutions, the ions $(C_{12}H_{21}O_{11}Ca)^+$ or $(C_{12}H_{21}O_{11}Ca_2)^{3+}$ are apparently present. After saturation of juice by carbon dioxide, which decreases the pH to 8.9–9.2, saccharates decompose. Formation of insoluble tricalcium saccharate is used to obtain saccharose from molasses. Similar complexes, as with calcium ions, are similarly formed with Sr^{2+} and Ba^{2+}ions.

Disaccharide lactose has a unique position in terms of its ability to form complexes. It was found that it forms complexes with metals (Ca, Ba, Sr, Mg, Mn, Zn, Na and Li) in the ratio of 1 : 1 and in the range of pH 2–6.5. Binding of calcium by lactose is considered to result in increased resorption of calcium from milk.

Polysaccharides derived from glycuronic acids (such as pectins and alginates) form strong complexes with Ca^{2+} ions, which result in the formation of gels. Non-utilisable insoluble polysaccharides (e.g. cellulose and some hemicelluloses) and lignin that are classified as the insoluble fibre can also bind various minerals. These interactions can have a significant effect on the resorption of bound elements in the gastrointestinal tract. When studying metal binding to cellulose and hemicelluloses (such as arabinoxylans) it was found that the strength of interaction decreases in the order: Cu > Zn > Ca. In slightly acidic media, cupric ions show a high affinity for cellulose, but in the presence of glycine the binding of copper to the surface of cellulose molecules is substantially reduced. Lignin has two types of binding sites for binding metals. For binding sites with high affinity, the interaction strength decreases in the order: Fe > Cu > Zn, while for binding sites with low affinity the order of decreasing interaction strength is: Cu > Fe > Zn.

Various components of dietary fibre also have considerably different ion-exchange capacity. A long-term excessive intake of dietary fibre may bring about symptoms of calcium, iron and zinc deficiency. This reduced resorption of minerals is especially pronounced for high doses of fibre and likewise of phytic acid.

6.2.2.4 Lipids

Food lipids usually contain only trace amounts of minerals. Non-polar triglycerols and waxes have virtually no possibility of bindig mineral components. The exception is the opportunity for π-interaction of unsaturated fatty acids and unsaturated lipids that are able to complex with transition metals, such as silver. The complexes are of the charge-transfer type where the unsaturated compound acts as an electron donor and the Ag^+ cation as an electron acceptor. Free fatty acids yield salts with metals.

The situation is different in the polar lipids as their molecules contain both hydrophobic chains of fatty acids and the hydrophilic residue. Phospholipids, especially phosphatidic and lysophosphatidic acids, form salts with various metal ions. The stability of salts of phosphatidic acid decreases as: $UO_2^{2+} > Th^{4+} > Ce^{3+} > La^{3+} > Cd^{2+} > Pb^{2+} > Mn^{2+} > Cu^{2+} > Zn^{2+} > Co^{2+} > Ca^{2+} > Mg^{2+} > Ni^{2+} > Sr^{2+} > Ba^{2+} > Ag^{+} > Li^{+} > Na^{+} > K^{+}$. Ampholytic phospholipids (e.g. phosphatidylserine and phosphatidylethanolamine) form complexes with metal ions. The phosphate group and probably the amino group are also involved in the metal binding.

[3]Carbonation is usually carried out in two stages, first at pH 10.8–11.9 and temperature of 60–70 °C for about 20 min and then, after another addition of lime milk (CaO content in the juice is 2–2.5%) at 80–85 °C for 30 min.

6.2.2.5 Carboxylic acids

Aliphatic mono- and dicarboxylic acids (such as acetic acid and oxalic acid, respectively), oxocarboxylic acids (such as pyruvic acid), hydroxycarboxylic acids (citric, tartaric, malic and lactic acids) and numerous aromatic acids (such as benzoic acid) are normal constituents of plant and animal foods. They form salts with metal ions and their anions also have the properties of ligands. Carboxylic acids are the major binding partners of metals in fruits and some vegetables. They also contribute significantly to the binding of minerals (metal ions) by humic substances in soils and sediments.

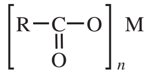
Calcium forms stable insoluble salt with oxalic acid (see Section 10.2.3.2). In plant cells with higher concentrations of oxalic acid, calcium oxalate can be actually present in the form of crystals. Some plants have been shown to bind metals in mixed complexes. For example, chromium can be bound in an oxalate–malate complex, and nickel and zinc can form a citrate–malate complex. Citric acid has been proven to be a low molecular weight zinc ligand in human milk, and in casein micelles it binds calcium. It is also used as a food additive (acidulant, synergist to antioxidants and sequestrant), so great attention has been paid to the formation of its complexes with metal ions. The addition to cereal products leads to increased solubility of naturally present iron, due to its release from phytic acid salts (phytates).

Other acidic substances, such as ascorbic acid, also form metals complexes. Of particular interest is the ternary complex of ascorbic acid with oxygen and Fe^{3+} or Cu^{2+} ions, formed during autoxidation of vitamin C (see Section 5.14.6.1.2). Ascorbic acid can also reduce metal ions. During the reduction of Fe^{3+} ions to Fe^{2+} ions, hydroxyl radicals are generated as byproducts (see Section 5.14.6.1.3). In this reaction, ascorbic acid acts as a prooxidant. The stability of ferric complexes of aliphatic organic acids decrease in the series: citrate $\gg$ succinate $\approx$ ascorbate $\approx$ malate > lactate.

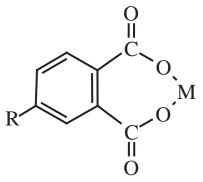
Examples of some complexes of aliphatic and aromatic carboxylic acids with metal ions are represented by formulae (**6-12**) to (**6-16**), and in Figure 6.4. Aromatic 1,2-dicarboxylic acids form metal complexes with M^{2+} ions in a molar ratio of 1 : 1 (**6-13**), but the metals may also be coordinated with a ligand belonging to two or more acid molecules (**6-14**).

6.2.2.6 Flavonoids and other plant phenols

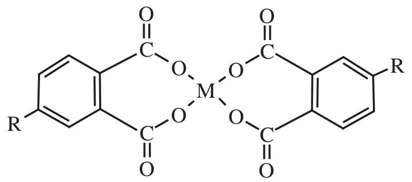
The transition element ions form complexes with a number of aromatic compounds in which two adjacent carbon atoms of the



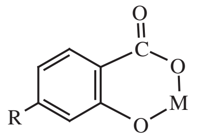
6-12, complex of metal (M) ion with aliphatic carboxylic acid



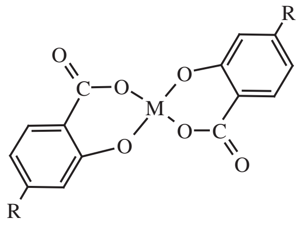
6-13, complex of metal (M) ion with aromatic dicarboxylic acid



6-14, complex of metal (M) ion with two molecules of aromatic dicarboxylic acid



6-15, complex of metal (M) ion with an aromatic 2-hydroxycarboxylic acid (salicylic acid derivative)



6-16, complex of metal (M) ion with two molecules of an aromatic 2-hydroxycarboxylic acid (salicylic acid derivative)

aromatic ring bear hydroxyl groups or hydroxyl and carbonyl groups. This structural motif is common to a large number of plant phenolic compounds.

Reaction with metal ions is related to the antioxidant activity of phenols. For example, flavonoids with two hydroxyl groups in positions C-3′ and C-4′, a carbonyl group in position C-4 and free hydroxyl at position C-3 or two hydroxyls in positions C-3 and C-5 have the highest antioxidant activity. The reaction of metal ions with flavonols is given in Figure 6.5 as an illustration, as flavonols belong to prominent yellow pigments of plants and act as very efficient antioxidants. Glycosides of flavonols, for example, have been identified as the main ligands of zinc in tea infusions. Aluminium in tealeaves is bound preferentially by catechins, and to a lesser extent by chlorogenic acid and other phenolics.

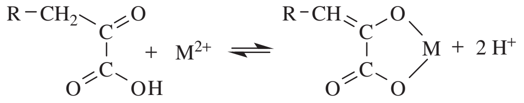


Figure 6.4 Formation of metal (M) ion complex of 2-oxocarboxylic acid.

6.2.2.7 Porphyrins and corrinoids

The macrocyclic tetrapyrrole skeletons of a range of natural substances can act as ligands that bind metals to form complexes. In foods, the main important types of macrocyclic tetrapyrroles are porphin (see Section 9.2) and corrin (see Section 5.13.1) derivatives.

Biologically important porphyrins (compounds with modified pyrrole subunits) are **haems** that contain iron as the central atom and act as a prosthetic group of important proteins, such as haemoglobin (the respiratory pigment in red blood cells of vertebrates), myoglobin (the pigment in muscle fibres) and other porphyrins. Metalloproteins that have haem as their prosthetic group are known as haemoproteins. Of the various Fe-porphyrins, haem *b* (an iron chelate derived from protoporphyrin IX) is the most important compound contained in the molecules of globins, catalase, peroxidase, as well as proteins involved in the synthesis and transport of nitric oxide and proteins involved in electron transport (P450 enzymes). Haems are also the prosthetic groups of a small number of enzymes, such as cytochrome *c* oxidase (haem a_3), cytochrome *c* 554 (haem *c*), nitrite reductase, sulfite reductase (sirohaem), cytochrome cd_1 sulfite reductase (hem d_1), quinol oxidase cytochrome *bo* (haem *o*) or hydroxylamine oxidoreductase (haem P460). With the exceptions of haem c and P450 that are covalently bound to the protein via the cysteine unit sulfur atom, other haems are bound to the corresponding proteins by non-covalent interactions, especially by hydrophobic interactions. This allows the substitution of hydrophobic porphyrin vinyl

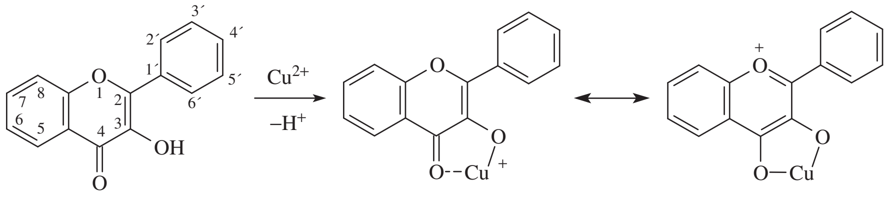


Figure 6.5 Complex of flavonols with Cu ions.

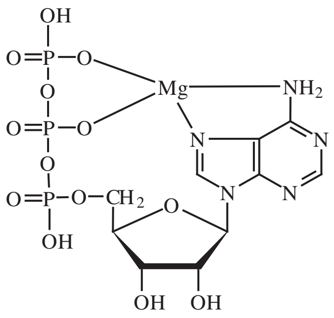
(majority of haems) and farnesyl groups (haem *a* and haem *o*). Iron bound to haemoproteins has the coordination number five or six. In addition to the four nitrogen atoms of the pyrrole subunits, iron is coordinated in a direction perpendicular to the plane of the porphyrin cycle with the imidazole nitrogen atom of the histidine residue, or with the sulfur atom of the cysteine residue, or with the phenolic oxygen atom of the tyrosine residue in the protein. The sixth coordination site of iron is usually occupied by a diatomic molecule, such as O_2 (e.g. in oxymyoglobin and haem oxidase), NO (e.g. in nitroxymyoglobin) or CO (e.g. in carbonylmyoglobin).

Porphyrin structures known as **chlorophylls** (green pigments of many plants, algae and cyanobacteria) contain magnesium as the central atom. Magnesium is not very tightly bound and in acidic media can be replaced by hydrogen ions. This substitution yields compounds called phaeophytins that can similarly re-bind other metal ions, such as Cu^{2+}, Zn^{2+} or Sn^{2+}. Some of these complexes are more stable than chlorophylls. Their spontaneous formation, however, proceeds only in contaminated foods. The intentional addition of salts of these elements to foods, in order to stabilise their colour, is not allowed. The most common form used as a food additive and in alternative medicine (including cancer prevention) is chlorophyllin (natural green 3, E140), a semi-synthetic mixture of water soluble sodium–copper salts derived from chlorophylls.

A group of cobalt-containing organometallic compounds derived from corrin that are known as cobalamins, have the activity of vitamin B_{12}. In addition to the four coordination bonds with nitrogens of the tetrapyrrole cycle, the central Co^{3+} atom is bound by two other coordination bonds. The fifth bond connects cobalt with the cyano group (in cyanocobalamin, the principal vitamin B_{12} form used in foods and nutritional supplements), water (in aquacobalamin also known as vitamin B_{12a}), methyl group (in methylcobalamin, methylvitamin B_{12}) or 5′-adenosyl group (5′-deoxy-5′-adenosylcobalamin, often called 5′-deoxyadenosylcobalamin or adenosylvitamin B_{12}). The sixth ligand can contain different groups or compounds, or this position may be not occupied at all (see Section 5.13.1).

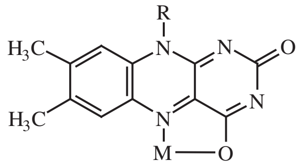
6.2.2.8 Other complexing agents

Phosphorylated organic substrates (such as phosphoric acid esters of sugars) have acidic properties and can form salts with various metals. Compounds containing heterocyclic nitrogen (such as nucleotides) have even greater binding abilities than sugar phosphates. For example, adenosine 5′-triphosphate (ATP) forms complexes with magnesium ions (**6-17**).

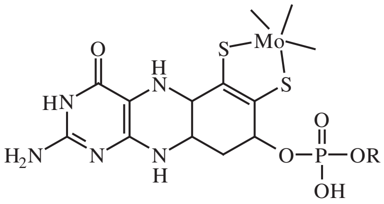


6-17, complex of magnesium with ATP

Similarly, some vitamins (riboflavin, folic acid) and derived compounds (riboflavin 5′-phosphate and flavoprotein FAD) have donor heteroatoms in suitable positions and thus have chelating properties. Isoalloxazine derived compounds, such as riboflavin, yield chelates shown in formula **6-18**. Comparable pyranopterins, containing two thiol groups, were identified as ligands of molybdenum and tungsten in cofactors of some bacterial metalloenzymes and metalloenzymes of mammals, such as cows' milk xanthinoxidase. Molybdopterin is a phosphorylated pyranopterin with an ene-dithiol group coordinating molybdenum. The structure of the molybdopterin complex can be seen in formula **6-19** (free valences of Mo are available for binding other pyranopterin molecules or other ligands, R represents hydrogen or adenosine).

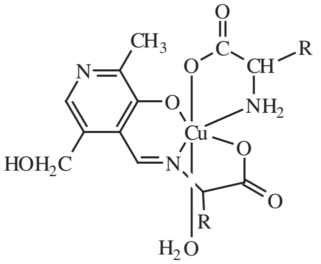


6-18, metal (M) complex of riboflavin and of other isoalloxazine derivatives



6-19, complex of molybdopterin with molybdene (R = H or adenosine)

Chelates can also be formed with amino acids and nicotinic acid or with amino acids and imines (such as reaction products of pyridoxal with amino acids) (**6-20**). Furthermore, metal ions form very stable chelates with β-dicarbonyl compounds (Figure 6.6). This property has been widely used in analytical chemistry.



6-20, copper complex of pyridoxal imine with an amino acid (R = amino acid residue)

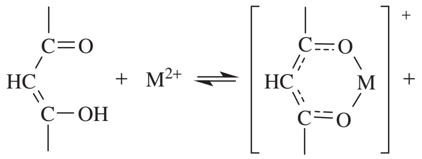


Figure 6.6 Complex of metal ions (M) with β-diketones (enolised).

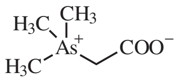
6.2.3 Bonding in covalent compounds

6.2.3.1 Compounds of non-metals and semimetals

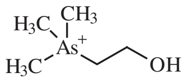
Covalent phosphorus compounds (sugar phosphates, phytic acid and nucleotides) have already been mentioned several times. Foods also contain many organic sulfur compounds, such as sulfur-containing amino acids, thiols, (oligo)sulfides, glucosinolates, heterocyclic compounds (derivatives of thiophene and thiazole). Their occurrence, properties, reactions and importance are covered elsewhere in this book.

Selenium is present in biological materials in a number of compounds that correspond in their structure to sulfur compounds. The amino acid L-selenocysteine (see Section 2.2.1.1.1) bound in proteins arises from selane and *O*-acetyl-L-serine, methylation yields Se-methyl-L-selenocysteine, reaction with *O*-succinyl-L-homoserine leads to L,L-selenocystathionine, hydrolysis of the last compound provides L-selenohomocysteine and methylation gives rise to L-selenomethionine (Figure 6.7). In plants growing in areas with a high level of selenium in the soil, the main compounds containing selenium are Se-methylselenocysteine, γ-L-glutamyl-Se-methylselenocysteine, selenocystathionine and selenomethionine. In many foods of plant and animal origin, the main compound of selenium is selenocysteine bound in proteins.

In biological materials arsenic can be found in a number of organic compounds. Methylarsonic acid, $CH_3AsO(OH)_2$ and dimethylarsinic (cacodylic) acid, $(CH_3)_2AsO(OH)$, are formed from inorganic arsenic compounds in aquatic organisms by biomethylation. These substances are also products of metabolic transformation of inorganic arsenic compounds that proceeds in mammals, so they occur in the urine of individuals intoxicated with arsenic. Seafood and some other marine organisms accumulate arsenic in their bodies in the form of quaternary arsonium compounds, such as arsenobetaine (**6-21**) and arsenocholine (**6-22**).



6-21, arsenobetaine



6-22, arsenocholine

In tissues of chondrichthyans (fish with a cartilaginous skeleton, such as sharks and rays), many species of marine fish (cod, flatfish, mackerel, herring, salmon and others), crustaceans (e.g. lobsters, shrimps and crabs) and molluscs (e.g. mussels and scallops), arsenobetaine is the main arsenic organic compound.

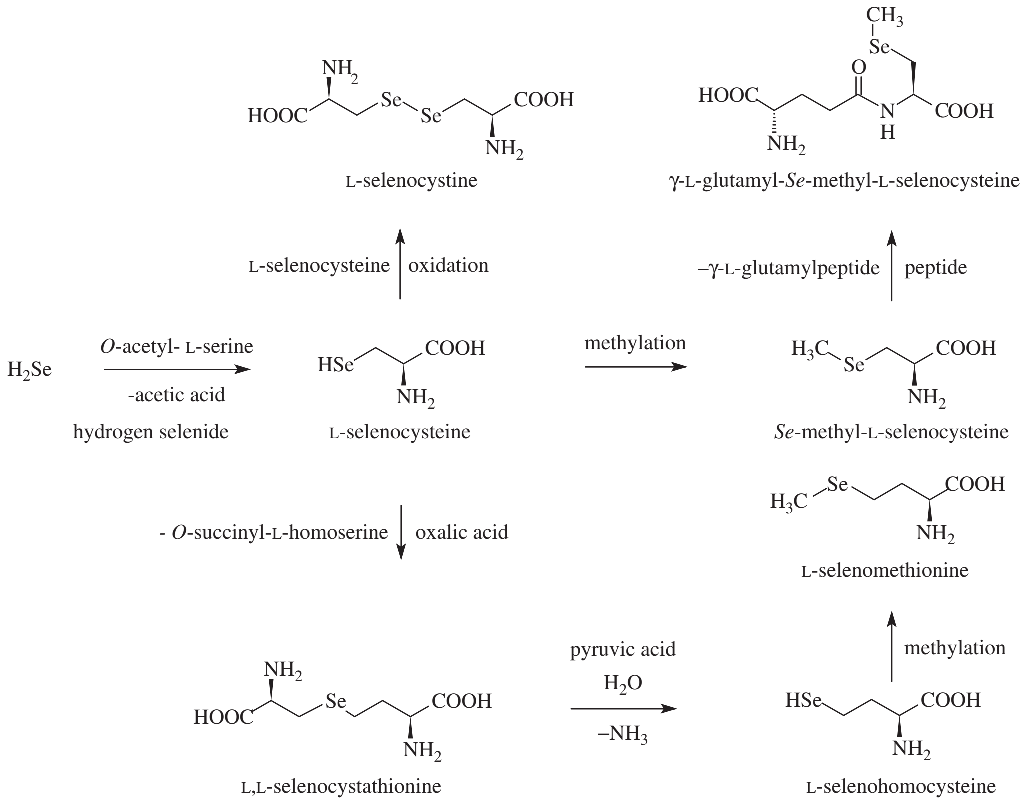
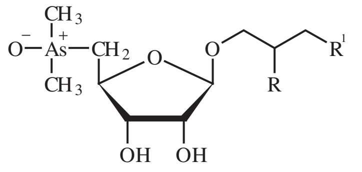
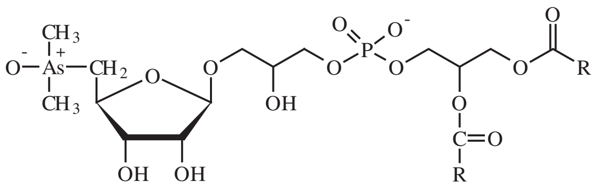


Figure 6.7 Biosynthesis of selenium amino acids.

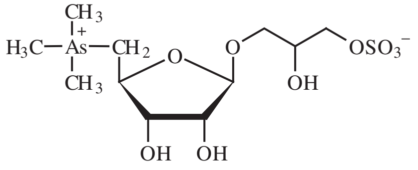
In shrimps, the dominant form is arsenocholine and arsenobetaine is present in lower amounts. In freshwater fish, arsenic is mostly found in further compounds that have not yet been satisfactorily described. Certain marine algae (such as *Ecklonia radiata*) contain **arsenosugars** derived from D-ribose, namely dimethylarsinoylriboside (**6-23**), the corresponding phosphatidylglycerol (**6-24**), a glycolipid usually esterified by palmitic acid, and trimethylarsinoylriboside glycerol sulfate (**6-25**).



6-23, dimethylarsinoylribosides: R = OH, R^1 = OH or R = OH, $R^1 = SO_3^-$ or R = OH, $R^1 = OSO_3^-$ or R= NH_2, $R^1 = OSO_3^-$ or R = OH, $R^1 = OPO_3^- OCH_2 CH(OH)CH_2OH$



6-24, dimethylarsinoylriboside phosphatidylglycerol: R = $[CH_2]_{14} CH_3$



6-25, trimethylarsinoylriboside glycerol sulfate

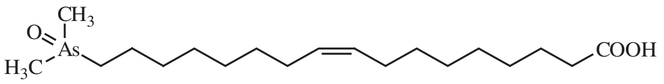
It has long been known that arsenic in seafood is contained in the lipid fraction. More recently, in cod liver oil containing 5 mg/kg arsenic, the following fatty acids containing arsenic were found: ω-(dimethylarsinoyl)alkanoic acids $(CH_3)_2As(O)(CH_2)_nCOOH$ ($n = 12$, 14, 16 and 18), ω-(dimethylarsinoyl)alkenoic acid with chain length of 17 carbon atoms and ω-(dimethylarsinoyl)alkapentaenoic acid with chain length of 21 carbons. The probable structures of these fatty acids are presented in formulae (**6-26**) and (**6-27**).

In marine fish oil (capelin, *Mallotus villosus*), some other lipophilic compounds containing arsenic have been found: two dimethylarsinoylalkanes, 1-(dimethylarsinoyl)pentadecane (**6-28**), 1-(dimethylarsinoyl)heptadecane and a polyene compound $(CH_3)_2As(O)C_{21}H_{30}$ with six double bonds. The position and geometry of double bonds is not yet known, but by analogy with the structure of docosahexaenoic acid, which commonly occurs in fish oil, it is assumed that this compound is all-*cis*-1-(dimethylarsinoyl)-heneicosa-2,5,8,11,14,17-hexaene (**6-29**).

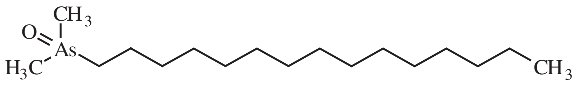
6.2.3.2 Organometallic compounds

The bonds of a metallic element to a carbon atom in organometallic compounds are relatively polar. Depending on the binding capacity of the metal, several bonds can be present, such as in tetramethyllead, $(CH_3)_4Pb$ (the main use of this was in antiknock additives for gasoline). Combustion of such fuel in car engines in the past was an important source of environmental contamination. Some alkyl groups may be replaced by other groups, as is the case of dimethyllead dichloride $(CH_3)_2PbCl_2$. Organometallic compounds with the full number of alkyl substituents are non-polar substances with relatively low boiling point temperatures. Apart from tetramethyllead, traces of some other synthetic organometallic compounds may be found in food as contaminants. These compounds, for example, include tetraethyl lead, $(CH_3CH_2)_4Pb$ (also used as an additive to gasoline), tributyl tin, a group of compounds containing the $(CH_3CH_2\ CH_2CH_2)_3Sn$ moiety as in tributyltin hydride or tributyltin oxide (used as pesticides) and in their decomposition products.

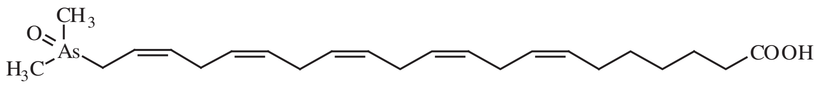
In bacteria, fungi, aquatic animals and plants, organometallic and organometalloid compounds are produced by **biomethylation** of some elements. This applies especially to mercury and arsenic, but to a lesser extent also to antimony, bismuth, selenium, tellurium, lead, tin and cadmium. Products of methylation of inorganic arsenic compounds are listed in the previous Section 6.2.3.1. Biological methylation of mercury can be carried out under aerobic or anaerobic conditions. A donor of methyl groups (CH_3 anion) is methylcobalamin that, in addition to mercury methylation, also participates in other methylation reactions (e.g. in the conversion of L-homocysteine into L-methionine). Other biomethylation agents are N^5-methyltetrahydrofolate and *S*-adenosyl-L-methionine that operate mainly in the methylation of arsenic compounds and other elements that form anions. The microorganisms methylating mercury include bacterial species of



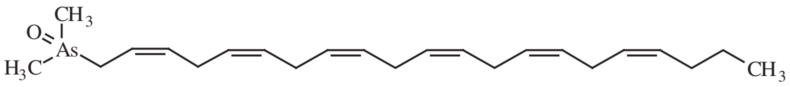
6-26, (Z)-17-(dimethylarsinoyl)heptadeca-9-enoic acid



6-28, 1-(dimethylarsinoyl)pentadecane



6-27, (7Z,10Z,13Z,16Z,19Z)-21-(dimethylarsinoyl)heneikosa-7,10,13,15,18-pentaenoic acid



6-29, all-*cis*-1-(dimethylarsinoyl)heneicosa-2,5,8,11,14,17-hexaene

the genera *Bifidobacterium*, *Chromobacterium*, *Clostridium*, *Enterobacter*, *Escherichia*, *Methanobacterium* and *Pseudomonas* and the microscopic fungi *Aspergillus niger*, *Neurospora crassa* and some other bacteria.

The product of biomethylation of the inorganic forms of mercury in fish and other aquatic organisms is called methyl mercury. This is the name given to a compound CH_3HgX, where X can be, for example, halogen, hydroxyl anion, a sulfhydryl group or a sulfide group. A minor product of mercury biomethylation and also a breakdown product of methylmercury sulfide, $(CH_3Hg)_2S$, is dimethylmercury, $(CH_3)_2Hg$. Methylmercury is the predominant form of mercury in fish, crustaceans and molluscs. Owing to the high affinity of mercury compounds for sulfur compounds, methylmercury CH_3Hg^+ cation bound through sulfhydryl groups of cysteine residues in proteins and peptides can thus be expected in fish tissue. It is also probable that this cation reacts with selenium compounds. In addition to fish, it is possible to find methylmercury in the bodies of animals that feed on fish, such as aquatic birds, cetaceans, pinnipeds and others, and in the gastrointestinal tract of various other animals. In this case, enteric bacteria cause the biomethylation of dietary mercury.

6.3 Essential elements

6.3.1 Sodium and potassium

6.3.1.1 Biochemistry and physiology

6.3.1.1.1 Occurrence in human body

The total sodium and potassium contents in the human body are about 70–100 g and 140–180 g, respectively. Sodium is found predominantly in the extracellular space, while potassium is located mainly inside the cells. Large amounts of sodium and potassium ions are found in the gastric juices.

6.3.1.1.2 Biochemical functions

The main function of sodium and potassium in the body, with chloride as the counter ion, is to maintain the osmotic pressure of fluids outside and inside cells and acid–base equilibrium. In addition, these elements are required for the activation of some enzymes, such as sodium for activatation of α-amylase and potassium for activation of glycolytic enzymes and respiratory chain enzymes. Potassium significantly affects muscle activity, especially the activity of cardiac muscle.

6.3.1.1.3 Metabolism

Resorption of sodium and potassium in the gastrointestinal tract is rapid and its effectiveness in a typical diet amounts to about 90%. The daily level of alkali metals obtained from food varies, with sodium ranging from 1.7 to 6.9 g and for potassium from 2 to 5.9 g. Both elements are excreted from the body in urine, but a significant amount of sodium is lost in sweat as well. Excessive sweating in extreme physical exertion may lead to loss of 8 g of sodium per day (which corresponds to 20 g NaCl). Unless sodium is supplied in the diet at an increased level, in such cases muscle cramps, headaches and diarrhoea develop. Although sodium is essential for the body functions, too much sodium can be harmful for people with kidney diseases and long-term excessive sodium intake can cause hypertension. Other sodium-related complications include oedema (noticeable swelling in legs, hands and face), heart failure and shortness of breath. Lack of potassium (caused, for example, by excessive loss of fluids in some diseases) can cause kidney failure, muscle weakness and an irregular heartbeat.

6.3.1.2 Occurrence in foods

In foods, sodium and potassium are found mainly as free ions. The natural sodium content in foods is highly variable. In many foods of plant origin, sodium is a minority element. In contrast, the potassium content in some plant materials is extremely high and can reach up to 2% (e.g. in tea and roasted coffee). The sodium content can increase by several orders in salted foods, to which it is added in the form of table salt for preservation and to increase flavour. The contents of sodium and potassium and other majority elements in selected foods are summarised in Table 6.4.

6.3.1.3 Nutrition

For an adult, the minimum required daily dose is 500 mg sodium and 2000 mg potassium; for children under 1 year old, 120–200 mg Na and 500–700 mg K; and for children 1–9 years old, 225–400 mg Na and 1000–1600 mg K. The actual amounts of sodium intake are often considerably higher. Approximately 75% of dietary sodium comes from sodium chloride or sodium hydrogen glutamate (monosodium glutamate) used in food technology and culinary processing. With the exception of manual workers, the amount of dietary sodium should not be greater than 2.4 g per day (6 g NaCl).

6.3.2 Chlorine

6.3.2.1 Biochemistry and physiology

The amount of chlorine in the human body is about 80 g. In living organisms, chlorine mostly occurs in the form of anions, which are, together with sodium counter ions, located in the cytoplasm of cells and extracellular fluids (blood, gastric juice and urine). Their main role is, similarly to sodium ions, to maintain osmotic pressure. In the gastric juices, chlorides act as counter ions of hydrogen

Table 6.4 Majority mineral element content in some crops and foods.

Food	Content (mg/kg)						
	Na	K	Cl	Mg	Ca	P	S
Pork meat	450-600	2600-4000	480-490	80-220	50-90	1300-2200	1400-2600
Beef meat	580-690	3400	400-740	170-250	30-150	1200-2000	750-2100
Chicken meat	460	4100	610	130-290	60-130	1200-2500	2700
Pork liver	770	3500	1000	220-260	60-70	3600-4800	2300-2800
Fish	650-1200	2200-3600	570-1200	140-310	60-5200	1900-3900	1400-2300
Milk (whole)[a]	480-500	1550-1600	900-980	110-140	1100-1300	870-980	290-330
Curd	-	1000	-	90	960-990	2000	1500
Cheese	450-14 100	1070-1100	12 000-23 000	170-550	1500-12 000	2900-8600	1900-2600
Yoghurt	660-770	1700-2200	-	140	1400	1100-1200	390-430
Egg (whole)	1350	1380	1600-1800	120-140	550-570	2100-2200	1700-2000
Egg (white)	1920	1480	1700	110	50-110	210-330	1800- 000
Egg (yolk)	500	1230	1400	140-150	1300-1400	5000-5900	1600-1700
Wheat	80	3500-5000	670	700-1500	230-500	3000-4100	1300-1500
Rye	20	5100	-	1100	240	3300	-
Flour (wheat)	20-30	1100-1300	360-480	210-1300	130-260	1000-3500	1300-1400
Bread (whole wheat)	4000-6000	2300-2500	9100	230-550	140-650	1800-2000	800-1000
Rice (peeled)	60	1000	60-270	260-430	50-110	770-1200	690-860
Peas (mature seeds)	20-380	2900- 900	390-600	1100-1300	440-780	3000-4300	1600-2000
Lentils	40-550	6700-8100	640	770	400-750	2400	1200
Beans	20-400	12 000	20-250	230-1800	300-1800	3700-4300	1100-1700
Soybeans	60	16 000	-	2400-2500	1300-1800	2900-7900	3500-3700
Cabbage	130	2300	220-450	120-230	300-750	280-680	440-900
Cauliflower	70-100	2100-4100	340	170	180-310	420-750	510-590
Spinach	600-1200	4900-7700	560-750	420-770	700-1250	250-550	270-400
Lettuce	30-100	2200	400	150-290	400-800	300-390	120-190
Tomatoes	30-60	900	500-600	110-180	60-140	210-260	110-140
Carrot	210	950	690	100-190	240-480	300-560	70-180
Peas (green)	20	3000	340-380	380-410	260-410	1000-1500	410-550
Onion	100-260	1300	190-270	70-160	200-440	300-480	360-530
Potatoes	30-280	4400-5700	450-790	200-320	30-130	320-580	240-350
Apples	16-30	900- 400	<10-190	35-70	30-80	100-130	30-100
Oranges	14-30	1800-2000	32-40	110-140	400-730	230-240	90-130
Bananas	10	3500	790	310-420	50-120	230-310	80-130
Strawberries	15-30	1500	180	120-170	180-260	230-350	80-140
Walnuts	30	6900	230	1300	600	4300-5100	1000
Tea (black)	450	21 600	5200	2500	4300	6300	1800
Coffee (roasted)	740	20 200	240	2400	1300	1600	1100
Milk chocolate	2800	3500	1700	590-710	2200-3200	2200-3000	780-1100

[a] Breast milk content of majority elements is: Na 160, K 530, Cl 860, Mg 20, Ca 250-310, P 130-160, S 100-160 mg/kg.

in hydrochloric acid, which is excreted by the gastric wall. The concentration of hydrochloric acid in the gastric juices is around 0.5% (pH 1–2). Chlorine is supplied in food mainly as sodium chloride at a level of 3–12 g per day. Chlorides are rapidly absorbed from the diet and excreted in the urine.

6.3.2.2 Occurrence in foods

Chlorine is a majority element in a range of foods. Chloride content in foods, however, depends mainly on whether table salt has been added to the raw material during food preparation and production. The concentrations of sodium and chlorides then correlate significantly. The chloride content of some foods are summarised in Table 6.4.

Perchlorates (ClO_4^-) are important oxidisers for fireworks, airbags and of solid rocket propellants. Low levels of perchlorates can then contaminate groundwater, drinking water and even milk. For example, levels of perchlorates up to 1.3 μg/l were found in California, USA (in 2004) in the milk of cows grazing on contaminated pastures. Perchlorates may interfere with the ability of the thyroid gland to utilise iodine in hormone production. Organic chlorine-containing compounds are generally classified as contaminants.

6.3.2.3 Nutrition

The minimum dose of chlorine required daily for an adult is 75 mg, for children under 1 year 180–300 mg, and for children from 1 to 9 years old 350–600 mg.

6.3.3 Magnesium and calcium

6.3.3.1 Biochemistry and physiology

6.3.3.1.1 Occurrence in human body

The content of magnesium in the body of an adult is about 25–40 g. This accounts for about 60% of the content of the skeleton. The highest concentrations of magnesium in soft tissues are found in the pancreas, liver and skeletal muscles. Blood and extracellular fluids contain only 1% of the total amount of magnesium in the body. Calcium is quantitatively the major mineral component in the human body. The total amount is about 1500 g, with 99% of this being in bones and teeth as calcium phosphate.

6.3.3.1.2 Biochemical functions

Both of these elements (magnesium and calcium) have many important biochemical functions in organisms. Magnesium is essential for all metabolic processes in which ATP arises or is hydrolysed, it participates in the stabilisation of the macromolecules of DNA and is required to activate certain enzymes, such as phosphotransferases (kinases) and phosphatases. In this function, magnesium ions can sometimes be replaced by manganous ions (Mn^{2+}). Owing to the binding of magnesium in chlorophylls, this metal is essential for photosynthesising organisms. Together with calcium, magnesium affects the permeability of biological membranes and cell excitability. The concentration of magnesium ions in the extracellular fluids affects the function of nerve cells. Magnesium deficiency, especially with an excess of calcium, leads to increased irritation and *vice versa*. A very high excess of magnesium causes suppression of nerve activity. In addition to structural functions together with proteins **osteocalcin** and **osteonectin**, the main biological function of calcium is participation in nerve and muscle activities. Calcium is also essential for blood clotting. A series of metabolic processes are regulated by calcium ions through the calcium-binding serum protein **calmodulin**, which affects the activity of certain enzymes (such as adenylate cyclase and along with magnesium the activity of ATPase).

6.3.3.1.3 Metabolism

Resorption of magnesium and calcium from food takes place in the small intestine. The effectiveness of resorption of magnesium from the diet at the normal dose of magnesium in a healthy man is 40–50%. A higher proportion of magnesium is resorbed in diets that contain low amounts of this element. Excessive amounts are excreted from the body in urine. The degree of resorption of calcium from food is low (about 5–15%), and is highly dependent on the chemical form of the calcium and the composition of the diet. The degree of resorption of calcium from spinach (the predominant form is calcium oxalate), for example, is only 2–5%. However, the degree of resorption of calcium from wheat bread (which contains calcium mainly in phytin) is about 40%, and from cabbage (where the main forms are calcium salts of organic acids, especially in lemons) it is between 40 and 70%.

Phytic acid and some components of dietary fibre also reduce the resorption of magnesium and some other elements from the diet (especially iron and zinc). A higher protein content in the diet increases the resorption of calcium.

6.3.3.2 Occurrence in foods

The contents of magnesium and calcium in certain foods are summarised in Table 6.4.

6.3.3.3 Nutrition

The recommended daily dietary intake of magnesium is 50–70 mg for children under 1 year, 150–200 mg for children under 6 years, 350 mg for adult men and 300 mg for adult women. During pregnancy and lactation, the daily dose of magnesium should be increased to 450 mg. The recommended daily intake is 400–500 mg of calcium for children under 1 year, 800–1200 mg for older children and adolescents, 800 mg for adults and 1200 mg for pregnant and lactating women.

6.3.4 Phosphorus

6.3.4.1 Biochemistry and physiology

6.3.4.1.1 Occurrence in human body

The adult human body contains about 420–840 g of phosphorus, and 80–85% of this is found in bones and teeth. The main minerals

of bones are calcium, phosphorus and fluorine. The weight ratio of Ca/P in bones is probably equal to 2. The phosphorus content in various tissues is as follows: in blood about 400 mg/l, in muscles 1700–2500 mg/kg, in nervous tissue 600 mg/kg, in bones and teeth 22% by weight.

6.3.4.1.2 Biochemical functions

Phosphorus acts as an essential element in living matter in a number of functions associated with the structure of compounds containing this element. They are mainly structural functions, functions in energy metabolism and activation, regulatory and catalytic functions.

The phosphorus compounds can be found in a number of important biological structures (inorganic phosphates in bones and teeth, phospholipids in biomembranes). Hydrolysis of macroergic phosphates, such as ATP, GTP, phosphoenolpyruvate and creatine phosphate, allows the realisation of energy-demanding biosynthetic reactions. Conversely, in catabolic processes (oxidative phosphorylation, reactions of the citric acid cycle and glycolysis), the chemical energy gained from the substrate is stored in ATP. Reaction of the transfer of the phosphate group (phosphorylation) activates common substrates (such as glucose). Participation of phosphate in the regulation of metabolism depends on the conversion of inactive forms of some enzymes (such as glycogen phosphorylase or protein kinase) by phosphorylation. The allosteric activator of some enzymes (including protein kinase) is another phosphorus compound known as cyclic AMP. Some enzyme co-factors are also phosphates (thiamine diphosphate, FAD, FMN, NADH and pyridoxal phosphate). Phosphorus is also present in nucleic acids, which provide storage and expression of genetic information. Phosphorus compounds are therefore involved in virtually all major metabolic processes.

6.3.4.1.3 Metabolism

Phosphorus is resorbed in the small intestine, mainly in the form of the HPO_4^{2-} anion. Resorption and excretion of phosphorus partly depends on the calcium content in the diet and vice versa. If one of these elements is present in large excess, this increases the excretion of the second element. This indicates that the optimum weight ratio of calcium to phosphorus in the diet is between 1 : 1 and 1 : 1.5.

The degree of resorption of phosphorus is dependent on the composition of the diet (in particular the levels of phosphorus and calcium and the forms of phosphorus received) and the age and health of the consumer. Newborns resorb 85–90% of phosphorus from breast milk and about 65–70% of phosphorus from cows' milk. In older children and adults, the degree of resorption of phosphorus from the normal diet is about 50–70%, but may increase to 90% in the case of low doses of phosphorus.

As regards the resorption of various phosphorus compounds, it is known that the best utilisable compounds are salts and esters of *ortho*-phosphoric (*o*-phosphoric) acid, and somewhat less utilisable are salts of *meta*-phosphoric (*m*-phosphoric) acid and polyphosphates. Phosphorus in the form of phytic acid is resorbed at a level of from 20 to 50%, and the efficiency of absorption decreases significantly at higher doses of dietary calcium. Partial resorption of phytate phosphorus appears to be related to the fact that intestinal alkaline phosphatase of some animals also has phytase activity.

6.3.4.2 Occurrence in foods

6.3.4.2.1 Total phosphorus content

Phosphorus is found in most foods in concentrations above 100 mg/kg. The exceptions are refined fats and refined sugar, which contain only traces of phosphorus. Nuts, cheeses and other dairy products are rich sources of dietary phosphorus. For cows' milk, the ratio of Ca to P is about 1.2 : 1, while for breast milk the ratio is about 2 : 1. The absolute amounts of both elements in breast milk compared with cows' milk are lower. Many foods of plant origin with high concentrations of phosphorus contain considerable amounts of phytic acid and its salts, phytates, which are not very utilisable. The phosphorus contents in foods are summarised in Table 6.4.

6.3.4.2.2 Phytic acid

Phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisdihydrogen phosphate) occurs in a number of important crops, especially cereals, legumes and oilseeds. The main form is a mixed calcium and magnesium salt, which is called phytin. Phytate phosphorus has reduced biological utilisation and lower utilisation than other minerals (Ca, Mg and Zn and Fe in particular). The contents of phytic acid in some food materials and foods, and the ratio of phytate phosphorus to total phosphorus, are shown in Table 6.5.

In addition to cereals, legumes and oilseeds, which are characterised by a high content of phytic acid, there are plant products with a low amount of phytic acid (potatoes, artichokes, carrots, broccoli, strawberries, blackberries and figs) and crops that do not contain phytic acid (lettuce, spinach, onion, celery, mushrooms, apples, bananas, pineapple and citrus fruits).

Phytic acid is accompanied by a smaller amount of the partial esters of *myo*-inositol with phosphoric acid. Theoretically there are 63 ($=2^6-1$) acyclic and cyclic esters. In animal tissues, for example, more than 20 different esters have been shown. In lenses containing 0.49% of phytic acid, 0.07% of a mixture of pentakisphosphates and 0.01% of the mixture of tetrakisphosphates are present.

It is assumed that phytic acid and phytates in seeds of plants are used as a storage form of phosphorus and other minerals. Phytic acid has, however, numerous other biological functions in plant and animal organisms; for example, it acts as an antioxidant and anticarcinogen. Partial phosphoric acid esters, such as *myo*-inositol-1,4,5-trisdihydrogenphosphate and *myo*-inositol-1,3,4,5-tetrakisdihydrogenphosphate, for example, act in the regulation of the intracellular calcium level; *myo*-inositol-1,3,4,5,6-pentakisdihydrogenphosphate modulates the affinity of haemoglobin for oxygen; *myo*-inositol-1,2,6-trisdihydrogenphosphate inhibits blood coagulation (platelet aggregation) and has anti-inflammatory effects. *Myo*-inositol itself was once considered a member of the B group vitamins.

Table 6.5 Phytic acid and phytate phosphorus contents in some crops and foods.

Material	Phytic acid (g/kg)	Phytate phosphorus (%)	Material	Phytic acid (g/kg)	Phytate phosphorus (%)
Bread (whole wheat)	4.3–8.2	38–66	Soy flour (defatted)	15.2–25.2	87
Wheat	3.9–13.5	60–80	Lentil	2.7–10.5	27–87
Rye	5.4–14.6	38–46	Peas	2.2–12.2	37
Barley	7.5–11.6	66–70	Almonds	12.9–14.6	82
Oat	7.0–11.6	49–71	Peanuts	17.6	57
Maize	8.3–22.2	71–88	Walnuts	6.5–7.7	42
Rice unpealed	8.4–8.9	–	Cocoa	0.9	15
Rice peeled	3.4–5.0	61	Carrot	0.2–0.3	16
Soybeans	10.0–22.2	50–70	Potatoes	0.2–0.5	19–23

Under normal conditions (acidic or alkaline medium), phytic acid is not hydrolysed chemically, but may be hydrolysed enzymatically. The enzymatic hydrolysis proceeds in fermented cereal products such as bread and yields pentakis-, tetrakis-, tris-, bis- and monodihydrogenphosphates and possibly also free *myo*-inositol. In wheat and rye flour, the phytase of the grain is still partly active, and hydrolysis of phytic acid in dough is made by the action of this endogenous phytase (6-phytase) that hydrolyses the ester bond, predominantly in position C-6. Subsequent hydrolysis is achieved by the action of phytase of baker's yeasts and phytases of colon microorganisms. Microbial phytases preferentially hydrolyse the ester bond in position C-3. Partial hydrolytic cleavage of ester bonds of phytic acid occurs during baking. It was found that the production of bread from wheat flour can result in a loss of up to 70–85% of the phytic acid originally present. Table 6.6 illustrates the overall extent of hydrolysis of phytic acid during the fermentation and baking of cereal products. Activity of phytase also increases during germination of seeds. Particularly high activity is seen in phytase of germinating grains of wheat and rye and phytase of pea seeds. Losses of phytic acid during the cooking of legumes are caused mainly by leaching.

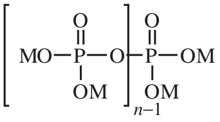
Phytic acid is also used as an agent (food additive) for the clarification of wines, which ensures the removal of ferric ions by precipitation of ferric phytate.

Table 6.6 *Myo*-inositol phosphates content in rice cakes.

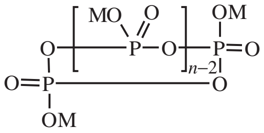
Phosphates of *myo*-inositol	Content (mg/kg)	Phosphates of *myo*-inositol	Content (mg/kg)
Hexakisphosphate	110	Trisphosphates	340
Pentakisphosphates	130	Bisphosphates	160
Tetrakisphosphates	460	Monophosphates	160

6.3.4.2.3 Phosphates and phosphoric acid

The phosphorus content in some foods can be raised above the natural level using food additives based on polyphosphoric acid salts or salts of phosphoric acid. Polyphosphates are either sodium or potassium salts of polyphosphoric acids with straight chains and different degrees of polymerisation, $M^{I}_{n+2}P_nO_{3n+1}$ (**6-30**) or salts of cyclic polyphosphoric acids, $(M^{I}PO_3)_n$ (**6-31**), which are actually oligomers of hydrogenphosphoric acid (*m*-phosphoric acid), HPO_3. The most commonly used materials are sodium and potassium phosphates, especially disodium dihydrogendiphosphate, tetrasodium diphosphate, sodium triphosphate and the so-called sodium hexametaphosphate.



6-30, linear polyphosphate (M = Na or K)



6-31, cyclic polyphosphate (M = Na or K)

The addition of phosphates (polyphosphates) to food affects the hydration of proteins and polysaccharides and their colloidal properties. It is used to increase the water-holding capacity (WHC) capacity, sometimes referred to as water-binding capacity (WBC) when water is added to cured meat and certain meat products. Phosphates also provide the appropriate texture to processed cheeses made from traditional cheese and emulsifying salts, often with the

addition of milk, salt, preservatives and food colouring. Polyphosphates are used as agents for clarification and stabilisation of wine and beer, and also have an antimicrobial effect. Phosphoric acid is often used as an acidifying agent (acidulant) for soft drinks (e.g. in Coca-Cola). In drinks in cans, phosphates retard the corrosion of the packaging.

6.3.4.3 Nutrition

The recommended daily dietary intake of phosphorus is 300–500 mg for children under 1 year, 800 mg for children under 10 years and 1200 mg for adults. These amounts are easily achieved in a normal diet. It is more important to maintain an appropriate ratio of calcium to phosphorus in the diet than the actual realised amount of phosphorus. Meat, poultry and fish (without bones) contain about 15–20 times more phosphorus than calcium; eggs, cereals and legumes contain about 2–4 times more phosphorus than calcium. Only milk, cheese, leafy vegetables and bones contain more calcium than phosphorus.

6.3.5 Sulfur

6.3.5.1 Biochemistry and physiology

The sulfur content in the human body is about 140 g. Foods contain a large number of covalent sulfur compounds. Many sulfur compounds perform important biochemical functions as biocatalysts (e.g. thiamine, pantothenic acid bound in coenzyme A and biotin), and sulfur-containing amino acids, cysteine and methionine, are protein constituents. Many sulfur compounds are important precursors of flavour-active compounds.

6.3.5.2 Occurrence in foods

The sulfur contents in selected foods are presented in Table 6.4.

6.3.5.3 Nutrition

The main sulfur compounds in foods are known and can be determined individually. Therefore, the total sulfur content in foods is not usually considered, and the recommended daily intake (approximately 0.1–0.6 g) is not officially established.

6.3.6 Iron

6.3.6.1 Biochemistry and physiology

6.3.6.1.1 Occurrence in human body

The total amount of iron in the body of an adult is about 3–5 g. The highest concentrations are found in the blood (haemoglobin), liver and spleen (ferritin and homosiderin); lower concentrations are in the kidney, heart and skeletal muscle (myoglobin). The concentration of iron in the pancreas and brain is about 2–10 times lower than the content of the liver or spleen. A list of the organic compounds of iron is given in Table 6.7.

Enzymes contain only minute quantities of the total iron in the body. They can be divided into two groups:

- haem enzymes: cytochromes, oxygenases and peroxidases
- non-haem enzymes: succinate dehydrogenase, also liver xanthine oxidase, NADH-cytochrome c reductase (flavin enzymes, oxidoreductases) and aconitase (lyase).

6.3.6.1.2 Biochemical functions

The function of iron in the body depends on the compound in which iron occurs. Iron mostly participates in oxygen transport through the bloodstream and oxygen storage in muscle tissue (the iron is in the haemoglobin and myoglobin) and participates in catalytic and oxidation–reduction reactions (iron in haem and flavin enzymes).

The blood plasma contains non-haem glycometalloprotein **transferrin**, which serves as a transport form of iron. Transferrin is pink coloured and contains Fe^{3+} bound to the apoprotein with a molecular weight of 86 kDa, which belongs to the group of β_1-globulins. A transferrin molecule consists of two identical subunits, each of which contains one binding site for iron.

Ferritin, together with **haemosiderin**, is the principal iron storage protein in bacteria, plants and animals. In animals, ferritins occur mainly in the spleen, liver and bone marrow. Ferritins are metalloproteins that can contain up to 23% iron. The protein constituent of apoferritin has a molecular weight of 445 kDa and consists of 24 cubically arranged subunits. Apoferritin forms the core package, which contains hydrated ferric hydroxide. One ferritin molecule can bind from 2000 up to 4500 iron atoms and

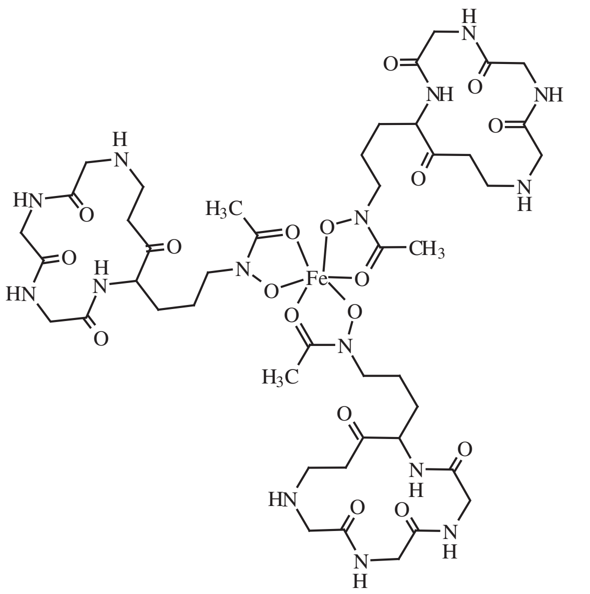
Table 6.7 Important iron compounds found in the human body.

Compound	Amount (g)	Amount in Fe (g)	Of total Fe (%)	Compound	Amount (g)	Amount in Fe (g)	Of total Fe (%)
Haemoglobin	900	3.0	60–70	Transferrin	10	0.004	0.1
Myoglobin	40	0.13	3–5	Catalase	5	0.004	0.1
Ferritin	2–4	0.4–0.8	7–15	Cytochrom c	0.8	0.004	0.1

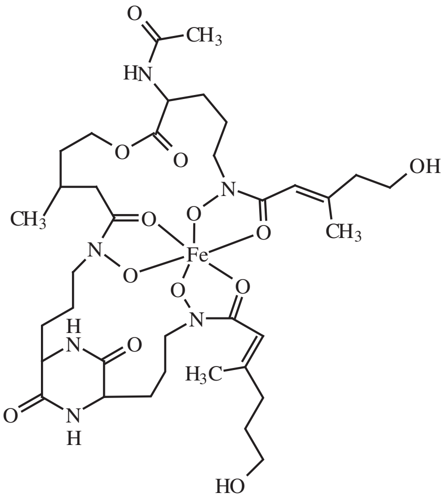
store them as Fe^{3+} ions. The binding of ferritin to iron reduces the risk of formation of insoluble iron compounds. In animals, ferritin is found not only inside cells, but is circulating in plasma if the body does not suffer from iron deficiency. Therefore, plasma levels of ferritin have sometimes been used as an index of iron storage deficiency. In some plants, a structurally similar metalloprotein called **phytoferritin** is present. The amino acid composition of the phytoferritins shows some similarities to those of mammalian apoferritins. Although the quaternary structure of 24 polypeptide chains is preserved, the phytoferritins can potentially store 1.2–1.4 times as much iron. Haemosiderin is an amorphous material, a complex of ferritin, denatured ferritin and other material, containing 35% iron in the form of ferric hydroxide. It is most commonly found in macrophages and is especially abundant in situations following haemorrhage, suggesting that its formation may be related to phagocytosis of red blood cells and haemoglobin. Haemosiderin can accumulate in different organs in various diseases.

Low molecular weight **siderophores** are excreted by many aerobic and facultative anaerobic microorganisms growing in environments with low iron content. Bacteria, such as pathogenic bacteria of the genera *Escherichia*, *Aerobacteria* and *Salmonella*, mainly contain catechol siderophores that are important for their acquisition of iron; aerobic and facultative anaerobic microorganisms (some yeasts and fungi) contain siderophores based on hydroxamic acids. A catecholate siderophore **enterobactin** (**6-32**) is primarily found in Gram-negative bacteria, such as *Escherichia coli* and *Salmonella typhimurium*. Examples of the second group of siderophores are **ferrichrome** (**6-33**) and **coprogen** (**6-34**) that are produced by fungi of the genera *Aspergillus*, *Ustilago* and *Penicillium*. Coprogen occurs in some microbially processed cheeses, as *Penicillium roqueforti* and *Penicillium camemberti* are used to ripen the blue and Camembert type cheeses, respectively.

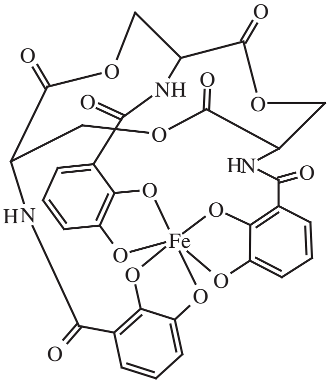
Other types of biologically important compounds are proteins with iron and sulfur. In these compounds, iron is bound to sulfhydryl groups of cysteine residues or even by sulfide ions. This group includes the FeS–proteins **rubredoxins** and Fe_2S_2-proteins and Fe_4S_4-proteins **ferredoxins**. These substances act as electron carriers by reversible change of the iron valency. Proteins with iron and sulfur are found in many organisms, for example in aerobic and anaerobic bacteria, algae, fungi, higher plants and animals.



6-33, ferrichrome



6-34, coprogen



6-32, ferric enterobactin

The main biological function of these proteins is electron transfer. An important non-haem metalloenzyme with bound iron, which occurs in prokaryotic organisms, higher plants and animals, is lipoxygenase, an enzyme catalysing the peroxidation of unsaturated fatty acids.

6.3.6.1.3 Metabolism

Iron resorption occurs predominantly in the duodenum and upper jejunum. The digestive tract resorbs 5–35% of iron present in a typical diet, depending on biological factors (health status, age and sex of the individual) and chemical factors (forms of dietary iron and diet composition). Its bioavailability decreases in the series:

haem > Fe^{2+} > Fe^{3+}. Deficiency of iron in the body can enhance the efficiency of its resorption in exceptional cases to 60%. Haem iron from foods of animal origin (haem pigments in blood and meat and in haemoproteins in the mitochondria) is resorbed more efficiently then haem iron in foods of plant origin (mitochondria). The mechanisms of haem iron resorption are completely different from those of inorganic iron (iron salts); the process is more efficient (10–20% of iron intake) and is independent of duodenal pH. Dietary inorganic iron can be absorbed in the form of divalent ions, which is easier than resorption of trivalent iron.

During the digestion of food, a partial reduction of trivalent iron to divalent iron occurs. The reduction of ferric ions to ferrous ions is partly due to some chemical components of partially digested food, but mainly to the ferric reductase enzyme (duodenal cytochrome *b*) immobilised on the outer membrane of the mucosal cells of the duodenum (enterocytes). The absorption of ferrous ions occurs in the duodenum and jejunum. The transport from the intestinal contents into the cells of the intestinal mucosa through the cell membrane is facilitated by a protein called divalent metal transporter, which transports all types of divalent metals across the enterocyte's cell membrane and into the cell. This transport is compensated by protons moving in the opposite direction. The early phases of haem iron resorption are different. Haem iron is absorbed by the intestinal mucosa as a porphyrin complex in the presence of a specific haem carrier protein 1 (HCP1) carrier. Within the mucosal cells, haem is transformed into biliverdin by haem oxygenase and carbon monoxide and Fe^{2+} ions are released. The cells of the intestinal mucosa (enterocytes) can store the absorbed iron in the form of ferritin, or can move it into the body. Before binding to apoferritin, ferrous ions require oxidation to ferric ions. Inside the enterocyte, the oxidation is ensured by the enzyme hephestin, which is a metalloprotein containing copper. Membrane transfer of divalent iron from enterocytes into the blood circulation is facilitated by a membrane protein called ferroportin. The amount of ferroportin channels in the membrane is controlled by a polypeptide hepcidin, which is synthesised in the liver. The efficiency of the overall process is diminished by the life cycle of the enterocytes and villi (lumenal surface finger-like projections covered predominantly with enterocytes), which last for only a few days (iron stored within the enterocyte then passes back into the intestinal contents).

Ferrous ions transferred into the blood are oxidised to ferric ions and then enter into the molecule of a transport protein under the catalysis of ferro oxidase ceruloplasmin, the main plasma metalloprotein containing copper. Transferrin ensures the transfer of iron to all tissues. Under normal conditions, about 30% of plasma transferrin is saturated with iron, and the remainder is apotransferrin. The target tissue captures transferrin by specific receptors and iron is immediately available for the synthesis of proteins and other haem metalloproteins or temporarily stored in ferritin. A substantial portion of the transported iron from transferrin is taken away in the bone marrow for the production of erythrocytes. New erythrocytes absorb the whole transferrin molecules. The release of iron from transferrin occurs due to a lower pH compared with the pH in the extracellular space. Apotransferrin is then released from the erythrocytes and iron is built into the porphyrin skeleton under the catalysis of ferrochelatase. The cells of the reticuloendothelial system that are located in the liver, spleen and bone marrow capture old and damaged erythrocytes. In these cells, the ferrous ions released from haem are stored in ferritin or haemosiderin, or released into the blood plasma through the ferroportin channel, where it is oxidised by ceruloplasmin to Fe^{3+} and again captured in transferrin.

Effective resorption is dependent not only on the iron valence, but can be enhanced or diminished in the presence of certain food components.

The most important substances that enhance iron resorption from the diet are:

- ascorbic acid, which acts as a reducing and chelating agent;
- organic acids (citric, lactic, malic, succinic and tartaric acids);
- amino acids, especially histidine, lysine and cysteine, which act as triple ligands and peptides and proteins composed of these amino acids;
- carbohydrates, which positively influence the retention of iron (efficiency decreases in the order: lactose, sucrose, glucose and starch).

The mechanism of this effect probably lies in the formation of complexes with iron that prevents the formation of insoluble forms of trivalent iron, especially $Fe(OH)_3$ and $FePO_4$, in the alkaline environment of the small intestine.

Most dietary factors negatively influencing iron resorption probably exert their action within the gastrointestinal lumen by making iron more or less bioavailable for resorption. Substances that reduce iron resorption either form insoluble iron compounds (phytic acid) or stable soluble compounds so that iron cannot be released for binding in proteins (ferritin) formed in cells of the intestinal mucosa. The diminished resorption of iron is mainly caused by tannins and phenolic compounds, phytic acid, fibre, higher doses of calcium and phosphorus and extremely high doses of trace elements (cobalt, zinc, copper and manganese).

Phenolic substances of tea are probably the most powerful factor that reduces iron resorption. Even in the presence of ascorbic acid, the resorption of iron is diminished due to the formation of insoluble complexes with tannins. Non-haem iron resorption is reduced by up to 62 and 35% when food is administered simultaneously with tea or coffee, respectively. In contrast, orange juice increases the resorption of iron by up to 85%.

There is no consistent information on the influence of fibre on the resorption of iron and other minerals. Many studies have focused on the effect of fibre added to food or food with a high proportion of fibre. Cereal fibre contains fairly high amounts of phytic acid, so in these cases it is not possible to distinguish between the effect of fibre and the phytate effect. When the effect of wheat fibre itself on iron resorption was observed in rats (experimental and control diet contained the same concentrations of phytates and various amounts of fibre), there were no differences in iron resorption. Fibre itself does not affect iron resorption, but the combined effect of fibre and

phytic acid is considerable. It illustrates, for example, low utilisation of iron from wheat germ, beans and lentils from foods with high contents of phytic acid. Fruit fibre does not contain phytic acid; therefore, reduced iron retention was found only when the highly esterified pectin fraction was removed from the apple pomace. Iron resorption is also negatively affected by certain proteins, such as soy protein and phosphoprotein phosvitin, which is present in egg yolk.

6.3.6.2 Occurrence in foods

The predominating form of iron in animal tissues is haem (particularly myoglobin and haemoglobin). In egg white, iron is bound in conalbumin, and in the yolk to phosphoprotein phosvitin. Milk contains the iron metalloprotein lactoferrin and part of the iron is bound to casein. Conalbumin and lactoferrin are structurally similar to serum transferrin. In plants, iron is bound in various complexes, especially with phytic acid, aliphatic hydroxycarboxylic acids, aminocarboxylic acids, thiols, phenolic substances, nucleotides, peptides and proteins. The iron content in selected foods is shown in Table 6.8. Foods rich in iron are offal dishes, meat, eggs, pulses, tea and cocoa. Moderate amounts of iron are found in fish, poultry, cereals, spinach, parsley and nuts. Low levels of iron are present in milk, dairy products, fats and oils, potatoes and most fruit.

Iron-deficiency anaemia is one result of the advanced-stage of iron deficiency. For food fortification with iron, many compounds of iron and elemental iron are used. In the evaluation of the biological value of the individual compounds, the increased level of haemoglobin (which cannot be formed in iron deficiency) in experimental animals on an iron dose is used as a criterion. Ferrous sulfate is used as a reference compound. Compared with ferrous sulfate, the usable forms of iron can be divided into three groups:

- good sources of iron with a relatively good biological value (over 70% compared with $FeSO_4$): ammonium ferric citrate, ferric chloride, ferric sulfate, ferric ammonium sulfate, ferrous fumarate, ferrous gluconate, ferrous sulfate, ferrous tartrate;
- sources of iron with a mean biological value (20–70%): ferric phosphate and elemental iron;
- sources with low biological value (<20%) iron oxide, ferric phosphate and ferrous carbonate.

In many countries, fortified milk products for infants contain ferrous fumarate. Other products that are often fortified with iron are cereal products.

6.3.6.3 Nutrition

The recommended daily intake of dietary iron is 6 mg for children under 6 months, 10 mg for children aged 6 months to 10 years, 12 mg for boys aged 11 to 18 years, 15 mg for girls and women from 11 to 50 years, and 10 mg for adult males and women over 50 years. The recommended dose for pregnant women is 30 mg and 15 mg during the lactation period. These values must be assessed with regard to the bioavailability of the iron. They were designed for healthy individuals, provided that the composition of the diet ensures good bioavailability of iron (the resorption efficiency of about 15%). This is only guaranteed by a diet containing a sufficient proportion of meat. The daily diet should provide an adult woman (weight of 60 kg) with about 2.6 mg of resorbed iron, and an adult male (weight of 80 kg) with about 1.4 mg of absorbed iron. Taking into account the very low bioavailability of iron from the diet of vegetarians (usually 5%), which does not contain haem iron and is rich in fibre, phytates and plant phenols, the total daily dose of iron for vegetarians should be about 50 mg for women and 30 mg for men.

Insufficient dietary intake of iron leads to a hypochromic microcytic anaemia and immune deterioration. In this type of anaemia, the amounts of haemoglobin and red blood cells are reduced, which results in reduction of transport of oxygen to the tissues and the decrease of organism performance. Severe anaemia can lead to heart failure. Iron deficiency can be divided into three stages. In the first stage, iron stores are reduced and decreased ferritin levels in plasma occur but functional changes are not noticeable. In the second stage, the proportion of protoporphyrin in erythrocytes increases and the haemoglobin level is slightly reduced (normal level of haemoglobin in plasma of men is 130–160 g/l and in females 120–160 g/l). Simultaneously, however, the total iron content in plasma, the ferritin content in plasma and tissue iron content strongly decrease. In the third stage, the symptoms of iron deficiency anaemia appear, tissue iron stores are depleted, which leads to a significant decrease in haemoglobin levels (hypochromia, also called hypochromasia or hypochromatism) and erythrocytes which are smaller than normal (microcytosis). Anaemia is more often encountered in women than in men and is often subject to inadequate nutrition, especially in developing countries. There are several other types of anaemia, produced by a variety of underlying causes.

Excessive iron intake by diet or dietary supplements (>1000 mg/day) and particularly in the event of a disorder of iron resorption regulation may cause accumulation of haemosiderin in the liver and other unwanted effects. This phenomenon is known as haemosiderosis and can lead to severe liver damage. The disorder of iron resorption known as hereditary haemochromatosis is a genetic disease. Transfusional iron overload can be the result of repeated blood transfusion.

6.3.7 Zinc

6.3.7.1 Biochemistry and physiology

6.3.7.1.1 Occurrence in human body

The adult human body contains from 1.4–3.0 g zinc, which is about a third to half the iron content, approximately 1–15 times the copper content and about 100 times the amont of manganese. Roughly half of the total zinc present is contained in muscles and about a third in the bones. High concentrations of zinc are found mainly in skin, hair, nails, eye, liver, kidney, spleen and male genital organs. In the cells of the liver, kidney and some other internal organs, zinc is bound in metallothionein.

Table 6.8 Iron and zinc contents in some crops and foods.

Food	Content (mg/kg) Fe	Zn	Food	Content (mg/kg) Fe	Zn
Pork meat	10–20	17–40	Beans	59–82	21–38
Beef meat	22–30	30–43	Soybeans	50–110	29–67
Chicken meat	4.3–8.4	8.1–12	Cabbage	3.1–9.0	1.5–2.9
Pork liver	130–370	56–112	Cauliflower	5.0–11	3.2–7.8
Fish	1.3–15	3.3–27	Spinach	10–40	4.3–13
Milk (whole)[a]	0.35–0.8	3.4–4.7	Lettuce	5.8–11	3.3–9.0
Curd	0.91–1.5	13–14	Tomatoes	2.2–5.0	1.2–4.8
Cheese	1.5–4.7	36–44	Carrot	3.4–7.4	2.5–5.9
Yoghurt	0.44–1.2	5.3–5.6	Peas (green)	18–22	11–15
Egg (whole)	21–26	13–15	Onion	3.0–6.1	3.1–5.2
Egg (white)	1.0–2.0	2.0	Potatoes	3.0–8.4	1.7–4.9
Egg (yolk)	61–72	38	Apples	2.3–4.8	0.2–4.9
Wheat	33–66	26–38	Oranges	1.3–5.0	0.9–1.2
Rye	25–28	22–40	Bananas	3.1–5.5	1.8–2.6
Flour (wheat)	12–25	8–36	Strawberries	3.6–9.6	1.1–1.9
Bread (whole wheat)	24–33	13–29	Walnuts	21–24	24
Rice (peeled)	6.0–23	10–15	Tea (black)	110–310	23–38
Peas (mature seeds)	47–68	20–49	Coffee (roasted)	41	6.1–8.0
Lentils	69–130	28–32	Milk chocolate	11–19	18–19

[a]The breast milk concentrations of Fe and Zn are 0.3–0.7 and 1.2 mg/kg, respectively.

The blood contains 6–7 mg/l zinc, with about 75–88% of this amount in erythrocytes, 12–22% in blood plasma (plasma zinc concentration is about 1 mg/l) and the rest is in the leucocytes and platelets. In blood plasma, the majority of zinc is bound to serum albumin and a smaller part to α_2-macroglobulin. The red blood cells contain zinc, mainly in the enzyme carbonate anhydratase.

6.3.7.1.2 Biochemical functions

Zinc is found in the bodies of all organisms. More than 200 metalloenzymes that contain zinc are known. The presence of zinc in molecules of some metalloenzymes is essential for their catalytic function. In other metalloproteins, zinc binding is involved in the fixation of the spatial structure of molecules (e.g. in protein transcription modulators, known as zinc finger proteins, with a specific bond to certain sections of DNA). The mammalian enzymes containing zinc are alcohol dehydrogenase, lactate dehydrogenase, superoxide dismutase, carboxypeptidases A and B and dipeptidase or carbonate anhydratase. Important zinc containing enzymes in the metabolism of yeast are phosphoglucomutase, phosphomannose isomerase, aldolase, alcohol dehydrogenase and pyruvate carboxylase. Bacterial zinc-containing enzymes include alkaline phosphatase, RNA-polymerase, DNA-polymerase and various proteinases. Zinc is therefore involved in the catalysis of reactions in many metabolic pathways. Zinc is also needed for the formation and activity of the pancreatic peptide hormone **insulin**. Human insulin is a peptide composed of 51 amino acids (5808 Da). The active form is a monomer. Six insulin molecules are assembled in a hexamer, and stored in the body by means of zinc ions bound by histidine residues.

6.3.7.1.3 Metabolism

Resorption of zinc in the digestive tract occurs mainly in the duodenum, but also in other parts of the small intestine. Resorption efficiency is normally about 30% and is regulated by intracellular ligands. In the transport of zinc into the intestinal cells (similarly to the iron transport) divalent cations are involved. In enterocytes, zinc is bound in metallothionein (at high doses of zinc in the diet) or in a complex with cysteine-rich intestinal protein (CRIP) (at lower doses of zinc). Resorption of zinc is higher in individuals with lower body weight and in cases of lower body saturation by zinc. In contrast, oral administration of high doses of zinc decreases the absorption efficiency.

The degree of resorption of zinc is dependent *inter alia* on the composition of the diet. A high content of proteins and amino acids increases the efficiency of resorption. Phytic acid and fibre operate in the opposite way. It was found that the molar ratio of phytate to zinc is a measure of the bioavailability of zinc from different foods. For example, bioavailability of zinc is good from a diet low in fibre and phytic acid (ratio phytate : Zn = <5), which is a diet with virtually no whole-grain cereal products. Mean utilisation (15–35%) is achieved in a mixed diet consisting of foods from plant and animal origins, where the ratio of phytate to Zn is approximately 5–10. Long-term intake of foods with a ratio of phytate to Zn greater than 20 leads to a zinc deficiency. Another criterion, which allows estimation of the bioavailability of zinc from food, is calcium content (molar content of calcium multiplied by phytate content and divided by zinc content). Diets with the value of this criterion higher than 3500 mmol/kg lead to slow growth of experimental animals (young rats). In human nutrition, at doses that ensure energy of 4.2 MJ (1000 kcal), the value of this criterion in the diet should not exceed 200 mmol/kg. Higher values mean a risk of serious deficiency. Table 6.9 shows the ratios of phytate/Zn and Ca × phytate/Zn for some cereal products. High calcium content decreases resorption of zinc by competing with zinc and, together with phytate, decreases zinc solubility in the intestinal contents. Zinc is excreted from the body via the faeces. It comprises both the zinc that was not resorbed from the diet and zinc expelled into the small intestine in secreted bile and pancreatic juice.

6.3.7.2 Occurrence in foods

The zinc contents of selected foods are shown in Table 6.8.

6.3.7.3 Nutrition

The recommended daily dietary intake of zinc is 5 mg for children under 1 year, 10 mg for children from 1 year to 10 years, 15 mg for boys and men, 12 mg for girls and women, 10 mg for men and women over 50 years. The recommended dose for pregnant women is 15 mg and 16–19 mg during lactation. In diets containing a high proportion of substances that deteriorate the bioavailability of zinc, the recommended amounts are not sufficient.

Zinc deficiency can occur during long-term acceptance of low-dose zinc diet or dietary components that reduce the bioavailability of zinc. This can be especially dangerous in childhood, where zinc deficiency results in slow growth and poor development of sexual organs in males. These effects were observed in some Arab countries, in cases where the dominant component of the diet is wholewheat bread prepared from unfermented dough. Other symptoms of deficiency can include loss of appetite, and changes in the skin, hair and nails. Many of these changes are reversible and administration of higher doses of zinc may restore the normal state.

Zinc is toxic at higher doses. Oral administration of more than 2 g Zn (as $ZnSO_4$) causes irritation of the mucous membranes of the digestive tract and vomiting. The adoption of such a high dose from food is entirely excluded. Long-term intake of amounts 10–30 times higher than the recommended daily intake (100–300 mg) leads to some changes in the blood count, which are typical for copper deficiency as zinc is an antagonist of copper.

6.3.8 Copper

6.3.8.1 Biochemistry and physiology

6.3.8.1.1 Occurrence in human body

The adult human body contains about 100–180 mg copper, corresponding to an average concentration of about 1.7 mg/kg body weight. The concentration of copper in the bodies of babies is substantially higher, at about 4.7 mg/kg body weight. Average concentrations of copper in different tissues and organs of the human body are as follows: liver 15 mg/kg (newborns up to 230 mg/kg), kidney 2.1 mg/kg, muscles 0.7 mg/kg, brain 5.6 mg/kg and lungs 2.2 mg/kg. In liver cells, most copper is bound in the molecules of the enzyme **superoxide dismutase** (relative molecular mass of 32 kDa). The brain tissue contains the metalloprotein **cerebrocuprein**.

The average concentration of copper in blood is 1.10 mg/l in men and 1.23 mg/l in women. More than 90% of copper in the body is found in the blood plasma. The major copper-binding substance in blood plasma is monomeric glycometalloprotein **ceruloplasmin** from a group of α_2-globulins (132 kDa), which is composed of 1046 amino acids and contains about 7–8% of sugar components. Ceruloplasmin has a blue colour and in the normal state one molecule of ceruloplasmin contains six atoms of copper (at full saturation additional binding sites may be occupied by an additional two copper atoms). Blood plasma contains about 300 mg/l of ceruloplasmin. In erythrocytes, copper occurs in another protein called **erythrocuprein** (31 kDa) and in the enzyme superoxide dismutase.

Table 6.9 Utilisable energy, calcium, zinc and phytic acid contents and values for phytate:Zn and Ca × phytate/Zn in wheat products.

Product	Energy (kJ/100 g)	Ca (mg/kg)	Zn (mg/kg)	Phytate (%)	Phytate:Zn (mol/mol)	Ca × phytate:Zn (mmol/kg)	Ca × phytate/Zn × 4.2 (mmol/MJ)
Wheat flour (white)	1520	170–240	10.2–16	0.21–0.34	14–29	86–138	24–38
Wheat flour (whole)	1418	340	29	0.68–0.92	23–31	194–264	57–78
Wheat germ	1506	390	123	1.64–2.24	13–18	129–185	36–49
Wheat bran	904	730	73	1.98–2.69	27–37	490–670	227–309

6.3.8.1.2 Biochemical functions

Copper is an essential trace element for humans and other animals. Copper ions are included in the active centres of many enzymes, especially cytochrome *c* oxidase, superoxide dismutase, various aminoxidases (such as lysyl oxidase), hydroxylases (e.g. dopamine β-hydroxylase and tyrosinase), galactose oxidase or different phenoloxidases, such as laccase and other oxidoreductases. The so-called blue copper proteins, for example plastocyanin, azurin and plantacyanin, occur in many prokaryotic organisms and plants. These proteins, by a change to the bound copper valency, provide electron transfer in various redox processes.

In some invertebrates, oxygen is transported in the blood by the cupric metalloprotein **haemocyanin** (oxyhaemocyanin). Haemocyanin contains two copper atoms (Cu^{+}) linked by three bonds with histidyl residues. One molecule of oxygen in oxyhaemocyanin forms a bridge between two atoms of Cu^{2+}. Cytochrome *c* oxidase is an enzyme that requires haem and copper. It catalyses the final reaction of the respiratory chain (the electron transport from the cytochrome system to an oxygen molecule):

$$O_2 + 4\ \text{ferrocytochrome}\ c\ (Fe^{2+}) + 4\ H^+ \rightarrow 2\ H_2O + 4\ \text{ferricytochrome}\ c\ (Fe^{3+})$$

The enzyme superoxide dismutase is essential for protecting subcellular structures from damage by oxidative reactions (free radicals), as it catalyses the disproportionation of superoxide anion radicals ($2O_2^{\bullet-}$), which are toxic products of oxygen metabolism (see Section 3.8.1.13):

$$2\ O_2^{\bullet-} + 2\ H^+ \rightarrow H_2O_2 + O_2$$

The resulting hydrogen peroxide is then decomposed in the presence of other enzymes (catalase, peroxidase). There are many different superoxide dismutases (SODs) and all are metalloenzymes. The most common enzymes are superoxide dismutases containing copper and zinc (CuZnSOD). Other superoxide dismutases include enzymes containing bound manganese (MnSOD), iron (FeSOD) or nickel (NiSOD). The change in the copper valence is essential for the catalytic activity of CuZnSOD that proceeds in two steps. Reaction of the oxidised form of the enzyme (Cu^{2+}) with the first molecule of superoxide produces oxygen and reduced enzyme (Cu^{+}), which reacts with the second molecule of superoxide yielding hydrogen peroxide and, simultaneously, the enzyme is reduced:

$$Cu^{2+}ZnSOD + O_2^{\bullet-} \rightarrow Cu^{+}ZnSOD + O_2$$
$$Cu^{+}ZnSOD + O_2^{\bullet-} + 2\ H^+ \rightarrow Cu^{2+}ZnSOD + H_2O_2$$

Some aminoxidases provide oxidative deamination of biogenic amines or amino acids. Lysyl oxidase is an enzyme essential for the integrity of connective tissue as it catalyses the post-translational deamination of lysyl or hydroxylysyl residues in proteins that subsequently form cross-linked **collagen** and **elastin** macromolecules. The lysyl residue reacts with oxygen, loses an ε-amino group and the terminal methylene group is oxidised to an aldehyde group. This allows the condensation of the aldehyde group with amino hydroxylysyl residue in the other chain to produce the corresponding imine. Amadori rearrangement of the imine produces a stable inter-chain bridge. This process leads to cross-linking of tropoelastin and continues to form desmosine and isodesmosine units (**2-98**) containing a pyridine nucleus and connecting the individual chains to form extremely resistant protein elastin.

Tyrosinase is an enzyme found in melanocytes (melanin-producing cells located in the bottom layer of the skin's epidermis) that participates in the pigmentation of skin and hair. This enzyme catalyses the hydroxylation of tyrosine to dopamine and two other reactions in the reactions sequence, leading to the main pigments known as **phaeomelanins** and **eumelanins** (see Section 9.3.1.1).

Another Cu-enzyme, α-amidating monooxygenase, is widespread in most higher animals. It provides oxidation elimination of the glycyl residue from the *C*-terminus of some peptide hormones (such as vasopressin, oxytocin, gastrin or calcitonin). At the same time the *C*-terminal amino acids are amidated. This reaction regulates the activity of the hormone. The original peptidylglycine reacts with ascorbic acid in the presence of oxygen with the formation of an intermediate peptidyl 2-hydroxyglycine. The final products are then deglycylpeptide amide, dehydroascorbic acid and glyoxylic acid.

Copper is also necessary for the efficient utilisation of iron and for the biosynthesis of some physiologically important compounds, such as the enzyme **ceruloplasmin**, which is produced in liver cells and acts in the blood plasma as the main Cu compound, but it is not involved in copper transport to the target organs. Plasma copper is transported primarily bound to albumin and partly in the form of complexes with low molecular weight ligands, such as histidine. Ceruloplasmin also has the catalytic activity of ferro oxidase, which means that it catalyses the oxidation of Fe^{2+} ions absorbed in the blood plasma to Fe^{3+} ions, thus allowing fixation of iron in the transferrin molecule. Copper deficiency, therefore, is similar to iron deficiency and leads to anaemia.

6.3.8.1.3 Metabolism

Copper is resorbed mainly in the duodenal part of the digestive tract of man. The degree of resorption is estimated at between 25 and 70%. Resorption of copper depends on the current saturation of the organism (its deficit leads to a higher degree of resorption). As a binding agent, metallothionein may play a role in the intake of copper by intestinal epithelial cells. The resorption of copper is achieved by two mechanisms: active transport, which prevails in the copper deficit in the body, and simple diffusion. Copper is excreted from the body primarily via the faeces, while the majority of the resorbed copper is excreted in the bile. If the daily dose of copper received in diet is approximately 2.5 mg, the amount resorbed into the blood circulation is about 1.2 mg. At the same time the liver eliminates in one day, via the bile, about 0.8–0.9 mg of copper into the intestine, and the kidney excretes about 0.1 mg of copper in urine. The real total daily intake of copper by the body is then only 0.2–0.3 mg, which is about 10% of the copper that is resorbed.

6.3.8.2 Occurrence in foods

Most foods contain less than 10 mg/kg copper. Milk is especially poor in copper, but the bioavailability of copper from human milk is very high. Higher concentrations of copper are found in the liver (especially in calf's liver), followed by legumes and some fungi. These concentrations are very variable. Copper content in selected foods is shown in Table 6.10. Copper naturally present in food is almost always linked in complex compounds with proteins and low molecular weight ligands. The level in foods can be, in exceptional cases, increased by contamination. This applies to crops (e.g. grapes or hops) treated with some pesticides based on compounds containing copper. Another possibility of contamination by copper may occur when copper containers for food processing of food raw materials are used (e.g. in brewing technology). Copper ions act as a catalyst in the oxidation of labile components of food (L-ascorbic acid and lipids).

6.3.8.3 Nutrition

The recommended daily dietary doses of copper are 0.4–0.7 mg for children under 1 year, 0.7–2.0 mg for children aged 1 to 10 years, 1.5–2.5 mg for adolescents and 1.5–3.0 mg for adults. Resorption of copper and its retention in the body depend on the chemical form in which this element is present in the diet. Experiments on laboratory animals have shown a higher utilisation of copper in the form of neutral and anionic complexes contained in plant material than in the form of copper sulfate. Availability of copper increases the presence of proteins and amino acids in the diet. Also, carboxylic and hydroxycarboxylic acids stimulate resorption of copper. In contrast, higher doses of ascorbic acid, fructose, molybdenum, sulfur compounds and zinc significantly reduce the resorption of copper. Ascorbic acid reduces cupric compounds to slightly soluble cuprous compounds. The effect of phytic acid and dietary fibre on copper resorption is, in comparison with the effect of these components in zinc, less pronounced.

Molybdenum and zinc are known antagonists of copper, so that high dietary doses of these elements can cause symptoms of copper deficiency. In the case of high doses of molybdenum, this is due to reduced resorption and increased copper excretion from the body in urine. Higher doses of dietary zinc lead to increased production of metallothionein in enterocytes, which leads to its retention in these cells, so that copper is released into the bloodstream to a lesser extent.

Copper deficiency in humans is very rare. The prolonged low intake of copper (0.8–1.0 mg daily) results in higher levels of cholesterol in the blood, changes in heart rhythm and reduced glucose tolerance. Experiments on animals have shown that copper deficiency causes serious disorders of iron metabolism and subsequently hypochromic microcytic anaemia. Other symptoms are movement disorders, changes in skin, hair, nails (impaired pigmentation and formation of keratin) and bones (facile fragility and deformation).

Copper toxicity to mammals is relatively low (LD_{50} value for oral administration of cupric sulfate, $CuSO_4{\cdot}5H_2O$, in rats is 300 mg/kg body weight). Copper ions are very toxic to fish.

6.3.9 Manganese

6.3.9.1 Biochemistry and physiology

6.3.9.1.1 Occurrence in human body

The adult human body contains about 10–20 mg manganese. Higher concentrations of manganese are found in the bones (2.6 mg/kg), liver (1.4 mg/kg), pancreas and kidney (1.2 mg/kg); lower concentrations (approximately 0. 2–0.3 mg/kg) are found in brain, spleen, heart and lungs and even lower in skeletal muscle (around 0.06 mg/kg). With regard to the subcellular level distribution, manganese is mostly present in the mitochondria. Biological structures containing keratin (such as bones and skin) are relatively rich in manganese. The manganese content in the blood is about 70 μg/l and according to some data only 8–10 μg/l. Manganese is mostly contained in blood erythrocytes, probably in the form of a porphyrin complex. In blood plasma, the majority of the trivalent manganese is bound to β_1-globulin.

6.3.9.1.2 Biochemical functions

The binding of manganese by most biologically important ligands is relatively weak, but manganese ions (Mn^{2+}) function as cofactors for a large variety of enzymes with many functions. This applies to oxidoreductases and other enzymes (some transferases, hydrolyses and lipases). Oxidoreductases catalyse oxidation and reduction (redox) reactions associated with the change of manganese valence (Mn^{II} versus Mn^{III} or Mn^{III} versus Mn^{IV}). The most important enzymes of the second group include pyruvate carboxylase and arginase. Both enzymes contain four molecules of Mn^{2+} ions. In the presence of biotin as a cofactor, pyruvate carboxylase catalyses the reaction: pyruvic acid + CO_2 + ATP + $H_2O \rightarrow$ oxaloacetic acid + ADP + H_3PO_4. Oxaloacetic acid is an intermediate of the citric acid cycle and carbohydrate biosynthesis (gluconeogenesis).

Arginase is an enzyme that catalyses the hydrolysis of arginine to urea and ornithine. This reaction represents the terminal phase of the urea (ornithine) cycle, which in mammals provides (in the form of urea) excretion of ammonia nitrogen released during decomposition of amino acids. The enzyme is composed of two subunits, each containing two atoms of Mn^{2+}, which are connected by two oxygen bridges. In the active centre, manganese atoms are fixed by coordination with four carboxylic groups of aspartic acid residues and nitrogens of the imidazole nuclei of two histidine residues.

Superoxide dismutases of some microorganisms (such as *Escherichia coli*) and some animals contain Mn^{3+}/Mn^{2+} ions instead of copper and zinc ions as the cofactor (see Section 3.8.1.13.2). Catalases of some microorganisms (e.g. the bacteria *Lactobacillus plantarum*) contain manganese instead of haem iron.

In addition to true metalloenzymes that contain manganese, there are a number of enzymes activated by manganese ions. These enzymes include various hydrolases, kinases, decarboxylases and glycosyltransferases. Manganese may also be bound, instead of magnesium, in enzyme molecules in which the catalytic function requires magnesium, while enzyme activity is maintained. In the enzyme glutamine synthetase, the bound manganese probably has

Table 6.10 Copper, manganese, nickel, cobalt and chromium contents in some crops and foods.

	Content (mg/kg)					
Food	**Cu**	**Mn**	**Ni**	**Co**	**Mo**	**Cr**
Pork meat	<0.4–1.8	0.12–0.18	<0.01–0.03	<0.001–0.012	<0.1	<0.01–0.09
Beef meat	0.6–1.8	0.10–0.14	<0.01–0.04	0.001–0.02	<0.1	<0.01–0.05
Chicken meat	0.35–0.51	0.14–0.16	<0.02–0.04	<0.01	<0.1–0.14	0.01–0.08
Pork liver	10–23	3.4–4.4	<0.01–0.28	0.002–0.023	2.0	0.003–0.16
Fish	0.2–3.1	0.10–3.1	0.005–0.05	<0.001–0.012	<0.1	0.002–0.23
Milk (whole)[a]	0.05–0.2	0.03–0.09	< 0.003–0.03	0.0004–0.0011	0.01–0.07	0.002–0.02
Curd	0.29–0.36	0.2–0.3	0.01–0.03	0.005	0.03–0.05	0.02
Cheese	0.3–19	0.4–0.8	0.02–0.2	0.01	0.05–0.1	0.01–0.13
Yoghurt	0.05–0.14	0.09–0.12	0.004–0.03	<0.005	<0.05	0.005–0.04
Egg (whole)	0.68–0.73	0.36–0.55	0.08	0.001–0.04	<0.05	0.005–0.02
Egg (white)	0.3	0.20	-	-	-	-
Egg (yolk)	1.6	1.0	-	-	-	-
Wheat	4.0–14	35–49	0.05–0.89	0.007–0.089	0.1–0.8	0.007–0.06
Rye	2.8–3.7	25.8	0.16	-	0.45	-
Flour (wheat)	2.0–6.5	7.3–36	<0.01–0.3	0.005–0.09	0.1–0.3	0.010–0.03
Bread (whole wheat)	3.5	13–21	0.08–0.2	0.01–0.05	<0.2	0.01–0.13
Rice (peeled)	0.6–2.8	5.3–15	0.1	0.01–0.02	0.1–0.3	0.01–0.03
Peas (mature seeds)	4.9–8.5	8.1–15	0.4–3.0	0.013–0.2	0.1–2.6	0.02–0.09
Lentils	5.8–8.9	12–14	2.3–3.0	0.016–0.092	2.0–10	0.048–0.054
Beans	6.0–13	12–20	2.5–5.0	0.01–0.3	1.0–3.0	0.05–0.10
Soybeans	8.0–20	14–90	2.0–10	0.05–0.14	<0.06–10	0.05–0.08
Cabbage	0.3–1.0	1.1–3.6	0.01–0.3	<0.001–0.01	<0.1	0.001–0.03
Cauliflower	0.41–0.64	1.5–3.9	0.03–1.0	0.001–0.01	<0.1	0.001–0.01
Spinach	0.6–1.7	3.5–34	0.05–0.4	0.001–0.02	<0.006–0.10	0.01–0.12
Lettuce	0.4–1.5	1.3–12	0.01–0.3	<0.001–0.006	<0.1	0.005–0.08
Tomatoes	0.4–1.0	0.7–1.6	0.01–0.25	<0.005	<0.005–0.09	0.002–0.01
Carrot	0.37–0.8	1.5–6.9	<0.01–0.09	0.001–0.005	<0.006–0.06	0.001–0.13
Peas (green)	1.9–2.4	3.4–4.3	0.2–0.7	0.002–0.01	0.2	0.005–0.04
Onion	0.35–0.91	1.1–3.8	0.03–0.42	0.001–0.01	<0.006–0.06	0.005–0.02
Potatoes	0.3–1.6	0.9–4.4	0.01–0.26	0.002–0.02	0.01–0.09	0.002–0.035
Apples	0.24–0.63	0.3–4.1	0.004–0.03	<0.001–0.005	<0.1	0.003–0.03
Oranges	0.44–0.91	0.3–0.5	0.01–0.04	0.001–0.01	<0.1	<0.001–0.02
Bananas	0.7–1.6	1.5–3.1	0.01–0.05	<0.001–0.002	<0.1	0.02–0.05
Strawberries	0.54–0.74	1.4–7.5	0.02–0.13	<0.001–0.01	<0.1	<0.002–0.02
Walnuts	3.1	18	9.0	0.008–0.29	<0.2	0.08–0.29
Tea (black)	11–33	320–1 040	1.9–12	0.60–1.0	0.13	0.62–2.6
Coffee (roasted)	8.2	15	0.6–1.0	0.34–0.88	<0.2	0.01–0.05
Milk chocolate	4.9	2.2–3.2	0.34	0.34	<0.2	0.04–0.1

[a]Breast milk concentrations of Cu, Mn, Ni, Co, Mo and Cr are 0.26–0.4, 0.006–0.03, 0.001–0.01, 0.0001, 0.002–0.01 and 0.0003 mg/kg, respectively.

a function of fixing the molecular structure and is not involved in the catalytic function.

Manganese is also important in the light reactions of photosynthesis in plant chloroplasts. The oxygen evolving complex is a part of photosystem II, occurring in the thylakoid membranes of the chloroplasts, which is responsible for the terminal photooxidation of water. Photosystem II is the oxidoreductase complex containing a cluster of four atoms of Mn and one Ca atom. Chlorophyll *P680*, which is present in this photosystem, absorbs light and transfers an electron to a pheophytin molecule from which it is transferred by other electron carriers to plastoquinone. This creates an electron hole that attracts electrons in the chlorophyll *P680* molecule. Chlorophyll *P680* thus acts as a strong oxidant ensuring, through changes in the oxidation state of the manganese cluster $CaMn_4$, oxidation of water (O^{2-}, respectively) to elemental oxygen. The reaction takes place as a cyclic action between five stages of transelectronase, which are referred to as S_0 to S_4. In the stage S_0, all four Mn atoms in the cluster are in the oxidation state III. Through four successive losses of electrons, the stage S_4 is then achieved, in which all four Mn atoms of the cluster are in the oxidation state IV. In the final phase of this cycle, two water molecules yield one molecule of oxygen and regenerated transelectronase is again in the stage S_0.

6.3.9.1.3 Metabolism

Resorption of manganese from food takes place in all parts of the small intestine. The efficiency of manganese resorption in adult humans is about 3–4%. In animal experiments it was found that resorption of manganese from food containing various manganese compounds with different solubilities (such as MnO, $MnCO_3$, $MnSO_4$ and $MnCl_2$) is very different. Although the mechanism of manganese resorption is not precisely known, there are some similarities with the metabolism of iron. Resorbed manganese is transported in blood plasma bound to transferrin. Part of manganese in plasma is also bound to albumin and α_2-macroglobulin. As with iron, binding of manganese to a transferrin molecule requires oxidation of divalent ions to trivalent ions, which is probably provided by ceruloplasmin. The concentration of manganese in the cytoplasm of primary cells is approximately 1×10^{-7} mol/l. Significantly higher amounts of manganese are found in some organelles.

Resorption of manganese is increased in the presence of low molecular weight ligands (such as citric acid or histidine). A high intake of iron in the diet can reduce the efficiency of manganese resorption. Also, high doses of calcium and phosphates reduce the bioavailability of manganese. In contrast, high doses of manganese reduce the resorption of iron and lead to a decrease in the haemoglobin level. There is also a competition in the resorption of manganese and cobalt.

In a diet containing low quantities of manganese and iron (e.g. in milk), the efficiency of manganese resorption is high. The resorption of manganese from breast milk is higher than the resorption of manganese from cows' milk. It probably relates to the different localisation of manganese in these fluids. In human milk, the majority of manganese is in the whey fraction, whereas in cows' milk, manganese is mostly contained in the casein fraction. The efficiency of manganese resorption from meat and fish is higher than from legumes.

The major route of excretion of manganese is through the bile excretion. The amount of manganese excreted in the urine is negligible.

6.3.9.2 Occurrence in foods

Food of animal origin contains low levels of manganese. Good sources are cereals and legumes. Fruits, especially some berries, have high concentrations of manganese. For example raspberries contain 6.7–18 mg/kg and blueberries 23–48 mg/kg manganese. Tealeaves and some spices have particularly high levels of manganese (e.g. cloves have manganese content of about 600 mg/kg, cardamom 320 mg/kg and ginger 160 mg/kg). The manganese content in selected foods is shown in Table 6.10. With regard to the chemical form of manganese in food, the major portion is in ionic form (especially as Mn^{2+}) or in the form of labile complexes.

6.3.9.3 Nutrition

Adequate and safe daily dietary doses of manganese are 0.3–1.0 mg for children under 1 year, 1.0–3.0 mg for children from 1 year to 10 years and from 2.0 to 5.0 mg for adolescents and for adults. Long-term deficiency in the diet can result in slow growth, abnormal bone development and impaired reproductive function. In neonates, deficiency may result in movement disorders. Owing to the influence of manganese on carbohydrate metabolism, deficiency may result in a reduced ability to synthesise and utilise glucose. Manganese deficiency in experimental animals causes a decrease in pancreatic insulin secretion and reduced insulin activity in peripheral tissues, as well as changes in activity of pancreatic amylase.

Toxic effects of manganese (growth retardation and anaemia) occur only at very high doses or long-term inhalation exposure. Poisoning by manganese from food is almost impossible. Chronic manganese poisoning, which was recorded in the miners working with manganese ore in dusty environments, leads to mental disorders and neurological symptoms similar to Parkinson's disease. Decreased blood pressure, elevated levels of calcium, bilirubin, cholesterol and total lipids in blood serum and a decrease of magnesium and protein levels may accompany inhalation exposure. The exposed individuals have a higher incidence of pneumonia.

6.3.10 Nickel

6.3.10.1 Biochemistry and physiology

6.3.10.1.1 Occurrence in human body

The body of an adult contains about 10 mg nickel. Data on concentrations of nickel in various tissues and organs of the human body are not entirely consistent. The nickel content can be influenced by many factors (such as age, gender, environment and smoking). The concentration of nickel in the lungs is 85 ± 65 μg/kg, kidney 10.5 ± 4.1 μg/kg, liver 8.2 ± 2.3 μg/kg, heart

$6.4 \pm 1.6\,\mu g/kg$ and bones $333 \pm 147\,\mu g/kg$. The normal concentration of nickel in the blood is about $5\,\mu g/l$ and in serum of about half of this. The serum nickel is bound to different proteins: albumin, HR-glycoprotein (histidine-rich glycoprotein), nickel plasmin (700 kDa), α_1-glycoprotein and in part also to the low molecular weight substances (mainly histidine).

6.3.10.1.2 Biochemical functions

So far there is no known specific biochemical function for nickel in animal organisms. In plants and microorganisms, some metalloenzymes containing nickel have been found. Such an enzyme is urease, found in soybeans, other legumes (this enzyme was first isolated from the seeds of the Jack bean, *Canavalia ensiformis*), rice and tobacco. Molecule of urease (580 kDa) contains 12 nickel atoms in six subunits. In the active enzyme centre, two nickel atoms are coordinated by four histidyl residues and one residue of ε-*N*-carbamoyl lysine, while the carbamoyl group and one molecule of water form a bridge between the two nickel atoms. Urease catalyses the hydrolysis of urea to ammonia and carbamate (carbamic acid), which is hydrolysed spontaneously to hydrogen carbonate (bicarbonate) and ammonium ions:

$$(H_2N)_2C{=}O + H_2O \rightarrow H_2N{-}COOH + NH_3$$
$$H_2N{-}COOH + H_2O \rightarrow HCO_3^- + NH_4^+$$

Some bacteria of the genera *Acetobacterium*, *Alcaligenes*, *Clostridium*, *Desulfovibrio*, *Methanobacterium* and *Vibrio* produce a variety of Ni-dependent oxidoreductases. Although no known animal enzymes containing nickel are known, the element can act as an activator of some enzymes. For example, calcineurin, which has phosphatase activity against phosphoproteins, is strongly activated by nickel ions. This enzyme contains bound iron and zinc and its activity is regulated by conformational changes that depend on the binding of other metal ions (Ni^{2+}, Mn^{2+} and Ca^{2+}) to some places in the molecule. A possible function of nickel is the participation in the resorption of iron. Nickel, through as yet unknown mechanisms, apparently facilitates resorption of iron by conversion of Fe^{3+} into Fe^{2+}.

Allergies to nickel are a phenomenon that has become increasingly important in recent years, largely because of the introduction of cheap fancy jewellery in which the underlying metal layer consists of nickel.

6.3.10.1.3 Metabolism

Most of the nickel contained in food passes through the gastrointestinal tract without resorption. The effective absorption of nickel is usually less than 10%. Increased resorption of nickel (20%) can occur if the saturation with iron is low. Resorbed nickel is excreted from the body mainly in urine. In the cytosol of renal cells, nickel is bound to a glycoprotein (15 kDa). The protein component of this glycoprotein contains a high proportion of glycine and no cysteine, so it is not identical with metallothionein. This glycoprotein also binds nickel in the urine.

6.3.10.2 Occurrence in foods

Fruits, cereals and foods of animal origin, with the exception of some seafood (oysters), have very low nickel content (hundredths to tenths of mg/kg). Higher concentrations are found in legumes, nuts, tealeaves, cocoa beans and cocoa products, such as chocolate. Refined vegetable oils and animal fats usually contain only trace amounts of nickel (around the thousandths of mg/kg) and of other transitional elements. Somewhat higher concentrations (hundredths to tenths of mg/kg) are found in margarine as solid fats may be manufactured from oils by hydrogenation in the presence of a nickel catalyst (a nickel–aluminium alloy known as Raney nickel), under controlled conditions. The concentration of nickel in food may increase during storage in metal containers, which has been shown in canned fruits. The nickel content in selected foods is shown in Table 6.10.

6.3.10.3 Nutrition

The recommended daily dietary doses of nickel have not been determined. Actual dose depends on dietary habits, and ranges between 150 and $700\,\mu g$/day. Animal experiments suggest that a deficit of nickel in the diet leads to slower growth.

Toxic effects of nickel occur only at levels $\geq$250 mg/kg food. Symptoms of nickel poisoning in animals are changes in fur and diarrhoea. High doses of dietary nickel are accompanied by changes of an increased number of erythrocytes, increased haemoglobin level and increased serum proteins in plasma, increased urea, iron, zinc, copper and nickel in the liver, increased hepatic activity of glutamate dehydrogenase and decreased activity of glucose 6-phosphate dehydrogenase. At the same time, the amount of iodine in the thyroid gland decreases. Nickel compounds are also irritating to the skin. Highly toxic and carcinogenic volatile nickel tetracarbonyl, $Ni(CO)_4$, used as a reagent in organometallic chemistry, is readily absorbed through the lungs.

6.3.11 Cobalt

6.3.11.1 Biochemistry and physiology

6.3.11.1.1 Occurrence in human body

The total amount of cobalt in the human body is <1.5 mg. The liver contains approximately 0.11 mg/kg of cobalt, skeletal muscle 0.2 mg/kg, bones 0.28 mg/kg, hairs 0.31 mg/kg, adipose tissue 0.36 mg/kg and blood about $0.3\,\mu g/l$ (mostly in plasma). The main biologically active compound of cobalt is vitamin B_{12}.

6.3.11.1.2 Biochemical functions

Cobalt is an essential element for bacteria, algae and ruminant mammals. For other organisms, including monogastric animals, cobalt is essential, in the form of the essential compound vitamin B_{12} (cobalamin). Rumen microflora of ruminants synthesises cobalamin from cobalt in the diet. Derivatives of vitamin B_{12} cobamides are cofactors of some enzymes, for example of methylmalonyl coenzyme A mutase, glutamate mutase and methionin synthetase.

6.3.11.1.3 Metabolism

In the gastrointestinal tract of humans, 20–97% of cobalt present in the diet is resorbed. For the resorption of vitamin B_{12}, a glycoprotein known as the gastric factor is essential, which allows the transport of cobalamin into cells in the intestinal mucosa. The effectiveness of cobalt resorption increases with iron deficiency. Cobalt is excreted from the body mainly in the urine.

6.3.11.2 Occurrence in foods

The richest sources of cobalt in the diet are beans and offal. Poor sources of cobalt are milk and dairy products, white flour products and sugar. Rich sources also include tea, coffee and chocolate. Cobalt content in selected foods is shown in Table 6.10.

6.3.11.3 Nutrition

The recommended dose of cobalt has not been established. Actual daily dietary doses are very low, being estimated at 5–10 μg.

6.3.12 Molybdenum

6.3.12.1 Biochemistry and physiology

6.3.12.1.1 Occurrence in human body

The total content of molybdenum in the human body is about 5–10 mg. The molybdenum content in the liver is approximately 0.36–0.9 mg/kg, in kidney 0.4 mg/kg and in brain and muscles 0.03 mg/kg. The molybdenum content in the blood fluctuates over a very wide range of concentrations (0.003–0.41 mg/l). Mean concentrations are 0.01–0.07 mg/l.

6.3.12.1.2 Biochemical functions

Molybdenum is an essential element for microorganisms and plants and even in animals several enzymes containing molybdenum have been found (such as xanthin oxidase, sulfite oxidase and dimethyl sulfoxide oxidase). Other enzymes containing molybdenum include formate dehydrogenase from *Escherichia coli* or arsenite oxidase from *Alcaligenes faecalis*. These enzymes contain molybdenum cofactor molybdopterin (**6-19**), a heterocyclic compound with a pyran ring fused to a pterin ring. The pyran ring is substituted by two thiol groups, which serve as ligands in molybdoenzymes (and tungstoenzymes). The *cis*-dithiolene group of the pyran ring binds the molybdenum, which is coordinated by three more (oxygen) ligands. Xanthin oxidase also contains, besides flavin adenine dinucleotide cofactor (FAD), two clusters of iron and sulfur in each of the two subunits. Xanthin oxidase catalyses the oxidation of hypoxanthine to xanthine and the oxidation of xanthine to uric acid (see Section 11.3.5):

$$\text{hypoxanthine} + H_2O + O_2 \rightarrow \text{xanthine} + H_2O_2$$
$$\text{xanthine} + H_2O + O_2 \rightarrow \text{uric acid} + H_2O_2$$

Xanthine oxidase is a very important enzyme in the catabolism of purines. In the congenital metabolic disorder called xanthinuria, xanthin oxidase is missing. The enzyme sulfite oxidase catalyses the oxidation of sulfites to sulfates using oxygen. It occurs in animals, plants and microorganisms. Dimethyl sulfoxide oxidase is capable of reducing dimethyl sulfoxide to dimethyl sulfide. This enzyme serves as the terminal reductase under anaerobic conditions in some bacteria. Formate dehydrogenases are enzymes that catalyse the oxidation of formate ($H{-}COO^-$) to hydrogen carbonate (HCO_3^-). Bacterial formate dehydrogenase H is known to use a selenium–molybdenum version of molybdopterin. Arsenite oxidase belongs to a sub-class of the dimethyl sulfoxide reductase family of molybdenum-containing enzymes. This enzyme also possesses a [3Fe–4S] iron–sulfur centre in the same sub-unit as the molybdenum centre, as well as an [2Fe–2S] centre in a separate sub-unit.

All organisms use the ammonia to manufacture amino acids, proteins, nucleic acids and other nitrogen-containing components necessary for life. Biological nitrogen fixation is the process that changes inert nitrogen from the atmosphere into biologically useful ammonia. This process is only mediated in nature by bacteria. Some plants benefit from nitrogen-fixing bacteria. In legumes and a few other plants, the bacteria live in small growths on the roots called nodules. Within these nodules, nitrogen fixation is carried out by the bacteria and the ammonia produced is absorbed by the plant. Among others, enzymes containing molybdenum, such as nitrogenases and nitrate reductases are involved nitrogen fixation. Nitrogenases ensure the conversion of elemental nitrogen into ammonia and nitrogen fixation in the soil. These enzymes are produced, for example, by some species of the genera *Rhizobium*, *Azotobacter*, *Clostridium* and *Klebsiella*. The overall reaction catalysed by nitrogenase proceeds according to the following equation:

$$N_2 + 8\,H^+ + 8e^- + 16\,\text{ATP} + 16\,H_2O$$
$$\rightarrow 2\,NH_3 + H_2 + 16\,\text{ADP} + 16\,H_3PO_4$$

Nitrogenases containing molybdenum are an intermolecular complex consisting of two parts called MoFe protein and Fe protein, respectively. The MoFe protein (230 kDa) has a heterotetrameric structure with an Fe–Mo cofactor and [Fe–S] cluster, while the Fe protein (64 kDa) has a homodimeric arrangement of sub-units bound by an [4Fe–4S] cluster. In some nitrogenises, molybdenum can be replaced by vanadium or iron.

Nitrate oxidase reduces nitrates (NO_3^-) to nitrites (NO_2^-). This enzyme occurs in microorganisms, algae, fungi and plants. The enzyme molecule is composed of two types of sub-units and contains, in addition to molybdenum and haem, non-haem iron. The electron transfer ensuring substrate reduction by cyclic changes in molybdenum valence is coupled with the cytochrome system. Reduction of nitrate to nitrite is the first phase of the denitrification process in which some microorganisms use nitrate ions as terminal electron acceptors instead of oxygen. The other phase of denitrification lies in the gradual reduction to nitric oxide, nitrous oxide and elemental nitrogen. Part of the generated nitrous oxide is released into the atmosphere and causes the greenhouse effect. All the enzymes that catalyse the individual reaction stages of denitrification (nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase) are metalloenzymes. The reverse process is nitrification, oxidation of ammonia nitrogen to

nitrate nitrogen. The nitrification bacteria involve bacteria of the genera *Nitrosococcus*, *Nitrosomonas*, *Nitrobacter* and *Nitrococcus*. The intermediates of nitrification are hydroxylamine and nitrite ions, minor byproducts include nitrogen oxides, hydrazine and molecular nitrogen. The processes of nitrification, denitrification and nitrogenation are, together with the consumption of nitrogenous nutrients by eukaryotic organisms and the catabolism of nitrogenous compounds, included in the nitrogen cycle in nature.

6.3.12.1.3 Metabolism

Molybdenum contained in food is resorbed in all parts of the small intestine and in part previously in the stomach. Resorption is quite effective (25–80%). With the exception of MoS_2, molybdenum is resorbed well from the soluble compounds (such as sodium and ammonium molybdates) and less soluble compounds (molybdenum trioxide and calcium molybdate). Resorption of molybdenum is competitively inhibited by sulfate anions. The excess of molybdenum is excreted in the urine.

Resorption, intermediary metabolism, excretion and physiological effects of molybdenum are strongly dependent on the interactions with sulfur compounds and their metabolites occurring in the diet. These interactions govern retention of molybdenum in various tissues and determine the tolerance to potentially toxic doses of molybdenum and molybdenum effects on the utilisation of copper. In the metabolic antagonism of molybdenum and copper, sulfur compounds mitigate the negative effects of higher doses of molybdenum.

6.3.12.2 Occurrence in foods

Relatively high contents of molybdenum are found in pulses (units of mg/kg). Moderate amounts are found in whole grain cereals and offal (tenths to units of mg/kg) and low amounts (<0.1 mg/kg) in most vegetables, fruits, fish, meat, milk, dairy products and fats. The molybdenum content in foods is presented in Table 6.10.

6.3.12.3 Nutrition

An adequate and safe daily dose of dietary molybdenum is 15–40 μg for children under 1 year, 25–75 μg for children from 1 year to 6 years old and 75–250 μg for individuals older than seven years. The actual daily dietary dose of molybdenum is in the range of about 120–240 μg. Symptoms of molybdenum deficiency are seen mainly in farm animals that live in areas with a low molybdenum content in the soils and vegetation.

6.3.13 Chromium

6.3.13.1 Biochemistry and physiology

6.3.13.1.1 Occurrence in human body

The total chromium content in the human body is estimated to be 5 mg, which is fairly evenly distributed. The concentration of chromium in individual tissues and organs, except the lungs, decreases with age. The normal content of chromium in blood plasma is 0.1–0.4 μg/l.

6.3.13.1.2 Biochemical functions

Chromium is a controversial element with important essentiality and toxicity, depending on its different species. Chromium in the oxidation state III is an important essential element. In contrast, hexavalent chromium compounds (chromates and dichromates) are toxic and may be responsible for certain allergenic, mutagenic and carcinogenic effects.

Trivalent chromium is an important metal in carbohydrate metabolism. It probably facilitates the interaction between sulfhydryl groups in cell membranes with the disulfide groups of insulin and thus allows insulin-stimulated utilisation of glucose. A complex compound of Cr^{III} with glycine, cysteine, glutamic acid and nicotinic acid, known as the glucose tolerance factor, was previously considered to be a physiologically active substance containing chromium. This substance, isolated from the yeast biomass, was able to increase glucose tolerance in experimental animals, but later it was shown that it was an artefact produced by acid hydrolysis of the chromium-binding substance chromodulin.

Today there are two known biologically active complexes of Cr^{III} with oligopeptides. The first compound, called **chromodulin**, occurs in the liver and kidney tissue of mammals, the second was isolated from cow colostrum. The oligopeptide **apochromodulin** (1.4 kDa), mainly containing glycine, cysteine, aspartic and glutamic acids, strongly binds four Cr^{III} atoms forming chromodulin. The peptide from colostrum has a similar amino acid composition. Both peptide complexes (but not apopeptides alone) stimulate glucose metabolism in adipocytes of rats. Chromodulin is incorporated into the amplification mechanism of insulin action. In the presence of insulin, chromodulin increases the activity of insulin receptors in adipocyte membranes. Apochromodulin is probably stored in the cells sensitive to insulin. The increase in plasma insulin level and subsequent insulin binding to insulin receptors in cells causes a conformational change that leads to phosphorylation of tyrosine residues on the inner side of the receptor. The Cr^{III} entry into cells and formation of chromodulin, which apparently interacts with insulin receptors, can stabilise the active conformation of receptors and increases their kinase activity.

Chromium compounds, through the potentiating of insulin effects and probably also by other mechanisms, also interfere with the metabolism of lipids and proteins. Increasing doses of dietary chromium partially reduce the levels of cholesterol and triacylglycerol in the blood plasma and simultaneously increase the proportion of high density lipoproteins (HDL). Chromium compounds are also involved in maintaining the structural integrity of nucleic acids as chromium protects RNA against thermal denaturation. It accumulates in the cell nuclei.

6.3.13.1.3 Metabolism

Inorganic chromium compounds are resorbed very little in the gastrointestinal tract of humans and other mammals. Resorption efficiency ranges from 0.4 to 3% and decreases with an increasing

dose of chromium (about 2% is resorbed at a dose of 10 μg/day and about 0.4% at a dose greater than 40 μg). Resorption of chromium is very fast. Even 15 min after administration, an increase in chromium concentration in the blood may be observed, and after 2 h in urine. The resorption efficiency of natural chromium complexes is probably much higher. Oxalic acid significantly increases and phytic acid decreases the resorption of trivalent chromium.

The resorption of hexavalent chromium compounds is 3–5 times higher than resorption of trivalent chromium compounds. In blood, hexavalent chromium enters into blood cells and erythrocytes more easily than trivalent chromium and binds to haemoglobin. Trivalent chromium is resorbed in the blood plasma bound to α-globulin and transferrin. Transferrin provides chromium transportation to the tissues. In some target tissues, chromium from transferrin binds to apochromodulin with the formation of chromodulin. In the cells, about 50% of chromium is contained in the nucleus and about 20% in the cytoplasm. Chromium is excreted from the body mainly in the urine. Excessive intake of chromium by experimental animals or chromium intoxication also leads to excretion of chromodulin in the urine. Biosynthesis of this metallopeptide thus participates in the detoxification of chromium.

6.3.13.2 Occurrence in foods

Data on the content of chromium in biological materials derived from the years 1950–1970 placed the chromium levels quite high, but these are now considered incorrect. Improved analytical techniques have helped to obtain the real chromium contents, which in some materials (including blood plasma and milk) is in the ultra trace region. Despite this progress in analytical methods, the results of different studies are contradictory. A summary of these results suggests a very wide interval of concentrations of chromium in various materials. During manufacture and storage, foods come into contact with metallic materials (such as stainless steel) and the initially very low content of chromium (several μg/kg) can be increased significantly due to contamination. A rich source of biologically utilisable chromium is brewer's yeasts. The chromium content in selected foods is shown in Table 6.10.

Under the assumptions that higher doses of chromium in the diet prevent diabetes and can to some extent increase weight loss, chromium compounds (usually complex of Cr^{3+} with picolinic acid) are often part of some food supplements. However, the effectiveness of such supplementation is still very controversial.

6.3.13.3 Nutrition

An adequate daily dietary dose of chromium is considered to be 50–200 μg. The actual dose of chromium found in several studies is in the range of 25 to 100 μg. In chromium deficiency, the following symptoms have been identified: impaired glucose tolerance, persistently elevated blood glucose level, elevated blood serum cholesterol and triacylglycerol levels and the presence of carbohydrates in urine. There is thus a link between chromium deficiency and the development of diabetes and cardiovascular diseases. Nerve and brain disorders have also been recorded in connection with the lack of chromium.

Toxic effects of Cr^{3+} compounds are manifested only at extremely high doses. Much more toxic are hexavalent chromium compounds, which cause growth failure and liver and kidney damage. Chromate contact with the skin may cause dermatitis. Chronic exposure to dust containing chromates increases the risk of lung cancer development.

6.3.14 Vanadium

6.3.14.1 Biochemistry and physiology

6.3.14.1.1 Occurrence in human body

The known levels of the transition metal vanadium in the human body are unreliable. Natural concentrations of vanadium in various tissues and organs are very low, but may substantially increase on exposure to vanadium compounds. For example, vanadium content in the kidney is in the range of 1–140 μg/kg, in liver 3–110 μg/kg, in muscles 10–110 μg/kg. The vanadium concentration in blood plasma is 0.02–1.3 μg/l.

6.3.14.1.2 Biochemical functions

As yet, the biological functions of vanadium have not been defined clearly. However, vanadium has an effect on the activity of important enzymes, especially the inhibition of (Na,K)-ATPase, other ATPases, phosphatases and phosphotransferases by metavanadate ions (VO^{3-}). Vanadium also stimulates the synthesis of cyclic AMP by adenylate cyclase activation and may therefore interfere with the metabolism of carbohydrates and lipids.

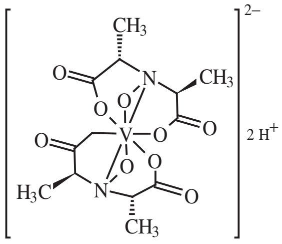
6.3.14.1.3 Metabolism

In the human gastrointestinal tract, only 0.1–1% of vanadium contained in the diet is resorbed. For some laboratory animals, however, fairly high resorption of vanadium from the diet with a predominance of casein and carbohydrates has been observed. Vanadium resorbed in the form of metavanadate ions (VO_3^-) containing V^V is reduced by glutathione in the blood to VO^{2+} ions that contain V^{IV} and form 2 : 1 metal–protein complexes with ferritin and transferrin. Transferrin then obviously provides the distribution of vanadium in the tissues. Vanadium is also present in a metalloprotein (metalloporphyrin) called **haemovanadin**. Excessive intake of vanadium is excreted in the urine.

6.3.14.2 Occurrence in foods

The vanadium content of most foods is extremely low. Milk, fats, vegetables and fruits contain <1–5 μg/kg of vanadium; whole grain cereals, meat, fish and liver contain 5–40 μg/kg. According to some sources, marine crustaceans and molluscs (e.g. oysters) have a higher content of vanadium, above 100 μg/kg. There is a high content of vanadium in some fungi of the genus *Amanita* (Amanitaceae) (36–250 mg/kg dry weight), such as the fly agaric

(*A. muscaria*), which concentrate vanadium to levels of up to 400 times those typically found in plants. These toxic toadstools contain a blue coloured complex (1 : 2) of V^{4+} cations with (2*S*,2′*S*)-*N*-hydroxyimino-2,2′-dipropionic acid, called **amavadin** (**6-35**).



6-35, amavadin

6.3.14.3 Nutrition

The recommended daily intake of vanadium has not been determined. The actual food intake of vanadium is estimated at 10–30 μg.

6.3.15 Selenium

6.3.15.1 Biochemistry and physiology

6.3.15.1.1 Occurrence in human body

The body of an adult contains about 15 mg of selenium. The highest concentrations of selenium are found in the kidney (0.2–1.5 mg/kg), liver (0.24–0.4 mg/kg), hairs (0.6–6 mg/kg) and bones (1–9 mg/kg), while muscles have a lower content (0.07–0.1 mg/kg). Concentrations of selenium in the blood range from 40 to 350 μg/l.

6.3.15.1.2 Biochemical functions

The essential importance of selenium was demonstrated in 1957 when selenium was detected in the so-called factor 3, which prevents liver necrosis in rats. In 1973, the first enzyme containing selenium, glutathione peroxidase, was identified. Selenium, as a part of glutathione peroxidase, enhances the biological effects of vitamin E. Glutathione peroxidase catalyses the reduction of hydrogen peroxide (H_2O_2) and hydroperoxides of fatty acids (R–O–O–H) using a tripeptide glutathione (see Section 2.3.3.1.1) that exists in reduced (G–SH) and oxidised (G–S–S–G) states:

$$2\ \text{G–SH} + H_2O_2 \rightarrow \text{G–S–S–G} + 2\ H_2O$$
$$2\ \text{G–SH} + \text{R–O–OH} \rightarrow \text{G–S–S–G} + \text{R–OH} + H_2O$$

The first step involves oxidation of the selenol of a selenocysteine residue (R–SeH) by hydrogen peroxide, which yields a derivative of selenenic acid (R–Se–OH). Selenenic acid is then converted back into selenol by a two-step process:

$$\text{R–SeH} + H_2O_2 \rightarrow \text{R–Se–OH} + H_2O$$
$$\text{R–Se–OH} + \text{G–SH} \rightarrow \text{G–S–Se–R} + H_2O$$
$$\text{G–S–Se–R} + \text{G–SH} \rightarrow \text{G–S–S–G} + \text{R–SeH}$$

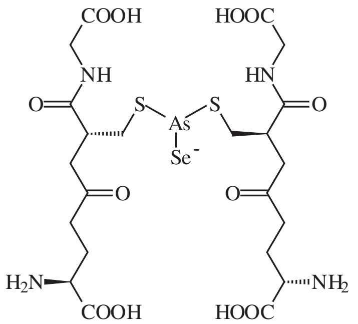
These reactions take place, for example, in erythrocytes and ensure removal of lipid hydroperoxides from damaged biological membranes (lipoproteins). Glutathione peroxidase thus provides protection against oxidative damage of biological structures. After removing the hydroperoxyl group, the hydroxylated lipids may be normally metabolised by β-oxidation. Reduced glutathione is regenerated by NADPH under the catalysis of glutathione reductase:

$$\text{G–S–S–G} + \text{NADPH} + H^+ \rightarrow 2\ \text{G–SH} + 2\ \text{NADP}^+$$

Glutathione peroxidase isolated from erythrocytes has a relative molecular weight of 85–95 kDa and consists of four subunits, each of which contains one selenocysteine molecule in its peptide chain.

Another selenoenzyme is iodothyronine 5′-deiodinase (also known as thyroxine 5′-deiodinase) from a subfamily of deiodinase enzymes important in the activation and deactivation of the thyroid gland hormones. There are two types of deiodinases: type I occurs in the liver, kidney, muscle and thyroid gland, type II is present in the brain, pituitary and adrenal glands. Iodothyronine 5′-deiodinase catalyses the conversion (deiodination) of the thyroid hormone thyroxine into the metabolically active triiodothyronine (see Section 2.2.1.2.5) and significantly enters into the metabolism of iodine and thyroid hormones.

A number of other selenium proteins have been discovered in biological materials, but their function is not yet sufficiently known. For example, a selenium protein (17 kDa) was found in rat sperm. It is interesting that high concentrations of selenium in male sexual organs are maintained in animals with selenium deficiency; therefore, selenium compounds are likely to be important for reproductive function. Some anticarcinogenic effects are also attributed to this element. Selenium also reduces the toxic effects of arsenic, cadmium, mercury, thallium and tellurium. With arsenic, the mechanism of this protective effect is the formation of selenobis(*S*-glutathionyl)arsinium anion, which is formed in erythrocytes and excreted in bile (**6-36**).

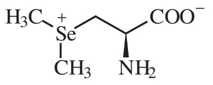


6-36, selenobis(*S*-glutathionyl)arsinium ion

6.3.15.1.3 Metabolism

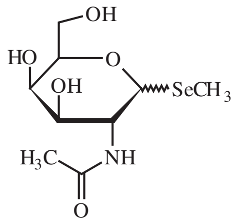
The effectiveness of selenium resorption in the gastrointestinal tract of humans is quite high, but depends on the selenium form. Organic and inorganic dietary selenium are both typically well absorbed through the intestinal membrane (70–95%). Since selenium and

sulfur have very similar properties, certain selenocompounds can be absorbed through the same pathways as their sulfur analogues. This is the case for selenate, which shares an active transport route with sulfate, and of selenomethionine, which passes the intestinal barrier using the same Na^+-dependant process as methionine. In contrast, selenite and selenocystine are taken up by a passive process (only in the direction of the concentration gradient) that is not affected by sulfite; however, selenocysteine does not seem to be influenced by Cys. Selenomethionine absorption is 95–97%, while selenite (SeO_3^{2-}) absorbtion is from 44 to 76%. Following their absorption, selenium species in the blood are partially accepted by blood cells and partially translocated towards different organs and tissues with the help of protein transporters (albumin and selenium-binding protein) in the blood plasma. It is suggested that ingested selenium is initially bound to albumin, which transports the element to the liver, where selenium is released and serves in the synthesis of selenoproteins. The most common selenoprotein is selenoprotein P, which is then released into the bloodstream to itself become a selenium transporter between the liver and various other organs and tissues. In the resorption and metabolism of selenium compounds, reduced glutathione (G–SH) and cysteine play an important role. Inorganic selenium compounds are metabolised in the body with the formation of selenotrisulfides (R–S–Se–S–R). The reaction of G–SH with selenite yields selenotrisulfide, known as selenodiglutathione (G–S–Se–S–G). With an excess of glutathione, unstable selenodisulfide (G–S–Se–H) forms and decomposes to selane (hydrogen selenide, H_2Se) and glutathione. Amino acids containing selenium are metabolised in methylation reactions. For example, methylation of selenocysteine gives *Se*-dimethyl-L-selenocysteine (**6-37**), which decomposes to L-alanine and volatile dimethylselenide $(CH_3)_2Se$. Methylation of dimethylselenide yields the trimethylselenonium ion $(CH_3)_3Se^+$.



6-37, *Se*-dimethyl-L-selenocysteine

Selenium is excreted from the body mainly in the urine (approximately 60%). Major selenium compounds in the urine are trimethylselenonium ions and selenosugars, such as 1,2-dideoxy-1-methylseleno-2-acetamido-D-galactopyranose (**6-38**). Part of the selenium is excreted by the lungs in the form of volatile compounds such as dimethylselenide, dimethyldiselenide and selane.



6-38, 1,2-dideoxy-1-methylseleno-2-acetamido-D-galactopyranose

6.3.15.2 Occurrence in foods

The average natural content of selenium in the earth's crust is 0.09 mg/kg, but it is distributed very unevenly. Selenium accompanies sulfur in nature, so it is a minor component of sulfides of copper, silver, lead and mercury. The highest concentrations of selenium are found in igneous and sedimentary rocks (in sulfide rocks at concentrations of more than 1000 mg/kg). In areas with geological sub-soil such as this, concentrations of selenium in the soil are elevated (30–300 mg/kg). Normal levels of selenium in the soil are 0.1–2.0 mg/kg dry matter. In natural waters only trace amounts of selenium occur. Concentrations of selenium in lake and river waters range from about 0.02 to 10 μg/l. Sea water contains 0.03 to 0.25 μg/l of selenium.

In some territories there are areas with high or low concentrations of selenium in the soil. For example, in China there are areas with both selenium deficiency and with extremely high concentrations of selenium. Mean concentrations of selenium are found in soils in large parts of the United States (in the states on the Atlantic and Pacific coasts). In Canada (excluding the province of Ontario), mean levels of selenium in soils also occur. By contrast, in some European countries (such as Finland, Switzerland and Czech Republic) and New Zealand, concentrations of selenium in the soil are very low. If the land is used for growing crops or grazing, the concentration of selenium in crops and animal bodies are characteristic of the region. Therefore, there are very significant regional differences in concentrations of selenium in agricultural products and foods. An overview of the selenium content in foods is given in Table 6.11.

The foods that are rich in selenium are mainly marine fish, crustaceans and molluscs (such as oysters and prawns), freshwater fish and offal (mainly kidneys). Eggs also have relatively high levels; most selenium is contained in the yolk. The content of selenium in milk, dairy products and meat is lower and is highly dependent on the animal nutrition. Concentrations of selenium in fruits and vegetables, except garlic, are very low (<0.02 mg/kg). Mushrooms contain selenium at the level of 0.03–1.4 mg/kg. The selenium content in foods of plant origin is fundamentally influenced by the selenium content in the soil or fertiliser used and its availability to the plant. The concentration of selenium in foods of animal origin is determined by the selenium content in the animal feed. Some pet foods are deliberately fortified with selenium compounds.

The level of selenium in the soil largely determines its concentration in plants. The ability of plants to absorb selenium depends on pH, moisture, oxygen and iron contents of the soil. The highest availability of selenium for plants usually comes from slightly alkaline aerobic environments. Hexavalent selenium compounds are very soluble in the soil. Tetravalent selenium compounds are less soluble, and their solubility decreases in the presence of ferric oxide. The vegetation in areas with a high content of selenium in the soil can contain up to tens of mg/kg of selenium in dry matter. Such a concentration can poison animals grazing on this vegetation. In addition, some plant species growing on seleniferous soils are selenium tolerant and accumulate very high concentrations of this element (selenium accumulators), but most plants are selenium non-accumulators and are selenium sensitive. Concentrations of

Table 6.11 Selenium content in major food materials and foods from different countries.

Foods	Content in mg/kg					
	USA[a]	Canada[a]	Venezuela	Finland[b]	Germany	Czech and Slovak Republics
Pork meat	0.04–0.24	0.31	0.83	0.01–0.09	0.19	0.02–0.07[d]
Beef meat	0.06–0.27	–	0.17	0.01–0.03	–	0.02
Chicken meat	0.10–0.12	–	–	0.08–0.14	–	0.07–0.11
Pork liver	0.64–0.70	0.36	0.36	0.34–0.51	0.17	0.09–0.34
Beef liver	0.43	0.50	0.69	0.03–0.13	0.09	0.02–0.14
Pork kidney	1.90–2.21	3.22	–	1.54–1.76	0.78	0.97–1.84
Beef kidney	1.45–1.70	2.31	–	0.62–0.78	0.95	0.20–1.02
Sweetwater fish	0.34–0.37	0.59	–	0.12–0.53	0.38	0.05–0.38
Sea fish	0.12–1.41	0.75–1.48	–	0.11–0.80	–	–
Milk (whole)[c]	0.06	0.15	–	0.001–0.004	0.20	0.003
Curd	–	0.07	–	0.02–0.03	–	–
Cheese	0.09	–	0.43	0.01–0.06	–	0.02–0.04[d]
Yoghurt	0.05	–	–	0.003	–	0.004–0.008[d]
Egg (whole)	0.10	0.39	1.52	0.02–0.16	–	0.18–0.24
Egg (white)	0.03–0.05	0.12–0.15	–	–	–	0.06
Egg (yolk)	0.13–0.18	0.13–0.69	–	0.30	–	0.53
Wheat	0.20–0.61	0.58–1.09	0.25	0.004–0.025	0.34–0.88	–
Rye	0.36	–	–	0.01	–	–
Flour (wheat)	0.18–0.52	0.28–0.64	–	0.010–0.12	–	0.016
Bread (whole wheat)	0.33–0.41	0.59–0.68	–	0.003–0.01	–	0.015–0.026[d]
Rice (peeled)	0.21–0.38	–	0.46	0.01–0.03	–	0.024–0.034[d]
Peas (mature seeds)	–	–	–	0.01	–	0.02
Lentils	–	0.61	–	–	0.10	0.03–0.08[d]
Beans	0.02–0.13	0.06	0.07	–	–	0.09
Soybeans	0.08–0.48	0.09	0.01	–	–	–
Cabbage	0.023	0.03	–	0.001–0.02	0.014	0.003
Cauliflower	0.007	0.004	0.01	<0.002	0.014	0.005
Spinach	0.012	–	–	<0.002	0.018	–
Lettuce	<0.001–0.011	0.008	–	<0.002	0.006	0.001[d]
Tomatoes	0.005	0.001	0.014	<0.002	0.007	< 0.001[d]
Carrot	0.022	0.006	–	<0.002	0.004	0.001–0.003[d]
Peas (green)	–	–	–	0.001–0.002	–	0.005
Onion	–	–	–	<0.002	–	0.003
Garlic	0.014–0.26	0.07	–	–	–	0.03–0.14
Potatoes	<0.002–0.055	0.023	0.016	0.001–0.002	0.017	0.003–0.018
Apples	0.005	0.004	0.006	0.001–0.003	0.01	0.001–0.003[d]

(*continued overleaf*)

Table 6.11 (*continued*)

Foods	Content in mg/kg					
	USA[a]	Canada[a]	Venezuela	Finland[b]	Germany	Czech and Slovak Republics
Oranges	0.013	0.015	0.008	<0.002	0.029	-
Bananas	0.01	-	0.005–0.06	0.001–0.01	-	-
Walnuts	0.08	-	-	-	-	-
Cocoa	0.21	-	-	-	-	-
Tea (black)	0.01–0.06	-	0.04	-	-	-
Coffee (roasted)	0.07–0.09	-	-	-	-	-

[a]Samples are from areas with a medium content of selenium in the soil.

[b]The table shows results from the 1970s, currently the levels of selenium in most Finnish foods are significantly higher due to the use of fertilisers with added selenium.

[c]The breast milk concentration of selenium is 0.006–0.028 mg/kg.

[d]Data from the Slovak Republic.

selenium in some species of the genus *Astragalus* from the Fabaceae family (commonly known as the legume family), for example in the selenium accumulating plant *A. bisulcatus*, commonly called two-grooved milkvetch or silver-leafed milkvetch, native to central and western North America, can reach thousands of mg/kg, and the plant can be toxic to cattle. Moderately selenium-accumulating plants belong to the Asteraceae family (commonly referred to as the aster, daisy or sunflower family). In most plants, the selenium level does not usually exceed 1 mg/kg.

In plants that strongly accumulate selenium, this element is present mainly in the form of free selenium amino acids and peptides. Selenium in foods is mainly in the form of selenium amino acids bound in protein molecules. In most plant foods, the major form of selenium is selenomethionine. For example, total selenium concentrations for Canadian lentils ranged from 0.16 to 0.72 mg/kg and almost all the selenium (86–95%) is present as selenomethionine with a small part (5–14%) as selenate, but the main components of garlic are selenocysteine and organic selenides. In foods of animal origin, the majority of selenium is bound in selenocysteine.

6.3.15.3 Nutrition

The recommended daily dietary doses of selenium are 10–15 μg for children under 1 year, 20 μg for children from 1 year to 6 years, 30 μg for children from 7 to 10 years, 40 μg for boys and 45 μg for girls from 11 to 14 years, 55 μg for adult women and 70 μg for adult men. For pregnant and lactating women, the daily dose should be increased to 65–75 μg.

The actual daily intake of selenium in the diet varies in different countries and depends on many factors. For example, the following amounts were determined: 330 μg in Venezuela, 130–200 μg in Canada, 80–130 μg in the United States, about 60 μg in Great Britain, in Finland 30–40 μg (in the 1970s) and in New Zealand 23–33 μg. For example, the mean selenium intakes in the United Kingdom have declined from >60 μg/day in 1974 to 29–39 μg/day by 1997 and in 2010 was estimated to be 48–58 μg/day. One reason for the decrease in selenium intake was that up until the mid-1970s approximately 50% of the wheat used to produce bread in the United Kingdom was imported from North America, particularly Canada, but by 1995 this import had fallen to <10% of the 1970 figure and in 2007/2008 82% of wheat for human consumption was grown in the United Kingdom, where the selenium content of food is low in areas where the selenium concentration in soil is low or where the selenium exists in chemical forms that are not readily available for the plants.

Owing to low levels of selenium in the blood serum of the population of Finland, at the beginning of the 1980s it was decided to increase the selenium content in food crops by the addition of sodium selenate to the fertilisers used (the doses were 6–16 mg of selenium per kg of fertiliser). In a few years (from 1984–1986), the content of selenium in important crops and livestock products had increased. For example, wheat selenium content increased from 0.01 to 0.23, in potatoes from <0.002 to 0.02, in milk from 0.008 to 0.03 and in eggs from 0.16 to 0.31 mg/kg. The average daily dose of dietary selenium increased to about 90 μg, and the selenium concentration in breast milk increased from 0.007 to 0.015 mg/kg.

The selenium content of foods, for example in the Czech Republic, is very low due to trace concentrations of this element in soil (0.07–0.12 mg/kg). There, the daily dietary intake was estimated at 25–40 μg. According to some sources, one third to half of population was in a state of mild to severe selenium deficiency. Average data from the last decade show some increase in levels of selenium in foods, so the intake (about 60 μg/day) is close to the recommended values. Importation of foods with high selenium contents contributed to the improved situation.

Selenium deficiency in animals is manifested by hepatic necrosis and a set of symptoms called white muscle disease. Very serious selenium deficiency in humans has been reported in China, in the Keshan region, where there is very low selenium content in the

soil. The heart disease known as Keshan's disease can be cured by increased doses of selenium.

Although selenium is an essential trace element, it is toxic if taken in excess. The symptoms of chronic selenium poisoning of animals (cattle, horses and sheep) have been known since the 19th century and are called alkali disease, because it was assumed that it was caused by drinking water with a high salt content. Poisoning in its first stage involves loss of hair, hoof deformities, and hoof and motion disorders. The second stage of poisoning (called Blind Staggers) causes blindness and often ends in the death of cattle.

Symptoms of poisoning in humans (selenosis) can occur even at doses that are 20 times the recommended daily dose (1–2 mg). Chronic human exposure to higher doses of selenium is manifested by inflammation of the respiratory tract, pulmonary oedema, bleeding, skin changes and depression. The characteristic garlic breath and metallic taste in the mouth is caused by the presence of dimethyldiselenide. In severe cases, jaundice, liver cirrhosis, hair and nails falling out, dental caries and kidney failure can appear.

6.3.16 Iodine

6.3.16.1 Biochemistry and physiology

6.3.16.1.1 Occurrence in human body

The body of an adult contains 10–30 mg of iodine. About 70–90% of iodine in the body is contained in the thyroid gland, a vitally important hormonal gland that plays an essential role in metabolism, growth and maturation of the human body.

6.3.16.1.2 Biochemical functions

Iodine is part of the thyroid hormones, iodinated aromatic amino acids derived from tyrosine that are known by their trivial names as 3,5,3′,5′-**tetraiodothyronine** (**thyroxine**, often abbreviated as T4) and 3,5,3′-**triiodothyronine** (also known as T3). Thyroid hormones regulate the rate of cellular oxidative processes that affect the consumption of oxygen in the liver, kidney and heart tissue, breakdown of lipids (lipolysis) and hydrolysis of glycogen to glucose (glycogenolysis), enhance resorption of glucose and galactose and affect thermoregulation. Activity of the thyroid gland is controlled by both the autonomic nervous system and the action of thyrotropin-releasing hormone (also known as thyroliberin or TRH). Thyroliberin is a tripeptide composed of 5-oxopyrrolidin-2-carboxylic acid, histidine and proline amide (362 Da) and produced in the hypothalamus, a small part of the brain. This hormone releases thyrotropin-stimulating hormone (also known as thyrotropin, thyrostimulin or TSH; α- and β-forms have relative molecular weight of 10.8 and 13 kDa, respectively) from the anterior pituitary gland, which in the thyroid gland influences the formation of thyroglobulin (Tg), a dimeric glycoprotein (660 kDa), which contains bound iodine.

6.3.16.1.3 Metabolism

In the form of iodide anions (I^-), which are the main form of iodine contained in foods, iodine is easily and completely absorbed in the gastrointestinal tract of humans. Other forms of iodine (salts of iodic acid iodates, IO_3^-) are first reduced to iodide ions. Iodine is rapidly transported by the blood to the thyroid gland, which captures about 60 μg of iodine in the form of iodide a day. During lactation, part of the iodine passes nto the milk and the rest is eliminated from the body in urine.

Synthesis of thyroid hormones has several stages. In the thyroid gland, iodine ions are oxidised to the active form (cation I^+) by the action of a specific thyroid peroxidase (thyroperoxidase), which reacts with tyrosyl residues of thyroglobulin to form 3-iodotyrosine. Subsequent iodisation of 3-iodotyrosine yields 3,5-diiodotyrosine. The condensation reaction of 3,5-diiodotyrosine with 3-iodotyrosine in the colloid of the thyroid follicle yields 3,5,3′-triiodothyronine. Two molecules of 3,5-diiodotyrosine combine to form thyroxine. These hormones, bound to thyroglobulin, are then released into the blood as a result of thyroglobulin proteolysis regulated by thyrotropin. In the blood, normal concentrations of 3,5,3′-triiodothyronine can vary by as much as 1–1.5 μg/l, and thyroxine concentrations range from 60 to 120 μg/l.

6.3.16.2 Occurrence in foods

The iodine content of most foods is in the tenths and hundredths of mg/kg. An overview of the iodine content in some common foods is given in Table 6.12. The content in foods of plant origin depends on the concentration of iodine in the soil. The highest concentrations of iodine were found in seafood and seaweed. The average concentration of iodine in seawater is about 60 μg/l and in sea salt 82 mg/kg. The level of iodine in animal products depends on the level of this element in feed, feed iodine supplements and the use of veterinary drugs containing iodine. The natural iodine content in milk and dairy products can also be increased through contamination from disinfection preparations containing iodine compounds. These products are used, for example, to disinfect the udders of cows and production equipment in dairies.

Certain food additives contain iodine. For example, potassium iodate and calcium iodate are components of preparations for stabilising dough. The synthetic red food colouring erythrosine contains 58% iodine (four iodine atoms in the molecule). Therefore, foods coloured using this pigment have a higher iodine content, but the bioavailability of erythrosine iodine is low (2–5%). The content of iodine in foods and meals may also increase with the use of table salt fortified with iodine (as sodium iodide or sodium iodate). The iodine concentration in table salt is 20–50 mg/kg.

6.3.16.3 Nutrition

The required daily dose of iodine, needed to prevent deficiency symptoms in adults, is estimated at 50–75 μg. To ensure some reserves, however, higher doses are recommended. The official recommended daily intake of iodine is 40–50 μg for children under 1 year, 70 μg for children 1 to 3 years old, 90–120 μg for children 4 to 10 years old and 150 μg for older children, adolescents and adults. During pregnancy, the recommended daily dose is 175 μg, and during lactation 200 μg. In several European and some other

Table 6.12 Iodine, fluorine, boron and silicon contents in some crops and foods.

Food	Content (mg/kg)			
	I[a]	F	B	Si
Pork meat	0.009–0.016[b]	<0.2	0.1–0.2	–
Beef meat	0.015–0.019[b]	0.1–0.2	0.1–0.2	1
Chicken meat	<0.005	<0.2	0.1–0.4	1
Pork liver	–	<0.2	<0.2	–
Sea fish	0.28–1.75	0.3–2.2	< 0.2	–
Milk (whole)[c]	0.016–0.75[b,d]	0.08–0.1	0.02–0.2	0.7
Curd	0.084–0.32[b]	0.2–0.4	0.1–0.2	–
Cheese	0.06–0.69	0.5–0.9	0.2–0.4	4–5
Yoghurt	0.022–0.26[b]	0.1	0.2	–
Eggs (whole)	0.029–0.73[b]	0.3	0.2–0.3	3
Wheat	0.024–0.043[b]	0.2–0.9	0.7–1.4	20–190
Flour (wheat)	0.017–0.025[b]	0.1–1.4	0.3–2.0	30–40
Bread (whole wheat)	–	0.4–0.8	0.3–1.0	30–50
Rice (peeled)	–	0.3–0.6	0.7–0.8	30–90
Rye	–	0.3–2.0	0.7–1.5	30–290
Barley	–	0.4–1.6	0.7–1.4	1400–2900
Oats	–	0.4–1.5	0.5–1.4	3400–6300
Peas (mature seeds)	–	0.3–0.9	6.1–7.1	20–50
Beans	–	1.0–2.0	14–26	50–60
Soybeans	–	0.9–1.3	28	30
Cabbage	<0.01	0.02–0.2	1.7–2.2	<2
Cauliflower	<0.005	0.02–0.2	1.7–2.2	2
Spinach	0.022–0.028	0.3–0.4	2.4–2.9	20–50
Lettuce	<0.01–0.018	0.02–0.4	1.3–1.8	12
Tomatoes	<0.01	0.02–0.1	0.8–1.1	<2
Carrot	0.013	0.03–0.2	2.4–4.0	1
Peas (green)	0.047	<0.1	2.6–3.4	2–18
Onion	0.025	0.04–0.1	1.3–3.3	5
Potatoes	0.018–0.037	0.06–0.2	1.1–1.8	4–6
Mushrooms	0.013	0.2–0.3	0.2–0.3	10–30
Apples	0.002–0.007	<0.1–0.3	1.0–6.0	2–5
Oranges	0.008	0.04–0.1	2.7–3.0	<2
Bananas	<0.005	0.1	1.4–2.2	50–80
Strawberries	0.09	0.03–0.3	1.7–2.1	10–20
Peanuts	0.11	–	18	50
Tea (black)	–	115–450	–	–
Chocolate (milk)	0.33	1.0	1.7–2.9	10

[a]Unless otherwise specified, data on the iodine content come from the United States.

[b]Data from the Czech Republic.

[c]The breast milk concentration of iodine is 0.06–0.18, of fluorine 0.016–0.04, of boron 0.06–0.08 and of silicon 0.7 mg/kg.

[d]In the United States, typical iodine concentrations in milk are 0.12–0.29 mg/kg, but some extreme values were also found (0.08 and 1.9 mg/kg).

countries, iodine deficiency is a significant public health problem. For example, in the Czech Republic, the average daily intake of iodine is about 100 µg. In a study in the United Kingdom published in 2011, almost 70% of test subjects were found to be iodine deficient.

Decreased thyroid gland function, called **hypothyroidism**, may be due to insufficient iodine intake or insufficient biosynthesis of hormones influenced by antithyroid agents also known as **goitrogens**. Iodine is necessary especially in the first 3 months of pregnancy, so that the nervous system of the baby can develop. Congenital hypothyroidism then manifests by cretinism. Hypothyroidism in young organisms leads to failure of growth, known as nanism or dwarfism (dwarf growth in which intelligence is not violated), and later to excessive enlargement of the thyroid gland. In the past, the thyroid gland enlargement occurred in inhabitants of the inland mountains, where the iodine content in food is low (e.g. in Switzerland). If the occurrence of such a disease is characteristic for certain areas, then we talk about endemic goitre.

Perchlorates (see Section 6.3.2.2) and some degradation products of glucosinolates, such as goitrin, isothiocyanates, nitriles and thiocyanate (rhodanide) ions (SCN^-), have goitrogenic (antithyroid) activities. Thiocyanate ions also arise in the body as detoxification products of cyanides. The other compounds that show antithyroid activity are some congeners of polychlorinated biphenyls (PCBs), some pesticides and a number of veterinary drugs containing thiourea residues (such as thiouracils, aminothiazoles and mercaptoimidazoles) that inhibit the enzyme thyroid peroxidase. These substances may be present in foods as contaminants (see Section 12.8.3.1).

Hyperfunction of the thyroid gland, called **hyperthyroidism**, manifests itself by production of an excessive amount of thyroid hormones. A disease where the thyroid gland is overactive is known as Graves' disease, whose symptoms are increased metabolism, body temperature and weight loss. Over-production of the hormone in childhood leads to giant growth (gigantism), while over-production in the later stages of growth leads to the growth of only the distal parts of the body (acromegaly).

6.3.17 Fluorine

6.3.17.1 Biochemistry and physiology

The human body contains about 0.8 to 2.5 g of fluorine. Fluorides are structural components of bones and teeth, and fluorine has a protective effect against dental caries. For healthy teeth development, adequate doses of fluorine are important, especially in childhood. In some countries, a sufficient amount of fluoride is supplied in drinking water. The optimal concentration of fluoride in water is about 1 mg/l. During teeth development, concentrations of fluorine in water higher than 10 mg/l lead to dental fluorosis in children. Teeth impacted by fluorosis have visible discoloration, ranging from white spots to brown and black stains. Fluorides present in food and drinking water are rapidly and efficiently absorbed in the gastrointestinal tract. Resorption efficiency is 85–98%. Resorption of fluorides, however, is significantly reduced in patients taking antacids (substances neutralising stomach acidity) based on aluminium oxide or aluminium hydroxide. In the body, fluorine is incorporated into hydroxyapatite, an inorganic calcium-containing constituent of the bone matrix and teeth. Under adequate dietary doses of fluorine, 50–70% of the ingested amount is excreted from the body in the urine.

6.3.17.2 Occurrence in foods

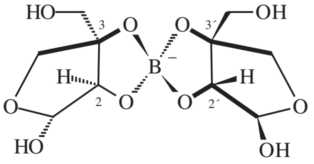
The fluorine content of most foods is in the tenths and hundredths of mg/kg (Table 6.12). Tealeaves have an extremely high fluorine content, so tea is the main source of fluorine in the diet.

6.3.17.3 Nutrition

The recommended daily dose of fluorine for children under 6 months ranges from 0.1 to 0.5 mg, for children from 6 months to 1 year this dose is 0.2–1.0 mg, for children from 1 to 3 years 0.5–1.5 mg, for children 4–10 years old 1.0–2.5 mg and 1.5 to 4.0 mg for adults. Insufficient intake of fluorine in the diet and drinking water increases tooth decay and can lead to osteoporosis. When long-term elevated fluoride doses (20–80 mg per day) are taken, symptoms of poisoning (fluorosis) appear, which leads to damage of the teeth, bones, kidney and nervous system as fluoride ions act as inhibitors of some enzymes.

6.3.18 Boron

Boron is a metalloid that occurs in nature mainly as borate minerals, such as borax, $Na_2B_4O_5(OH)_4{\cdot}8\ H_2O$, and borosilicates. In biological material, compounds of boron (boric acid and borates) form stable complexes with polyhydroxy compounds, such as sugar alcohols, sugars and substances derived from them (nucleotides, riboflavin and ascorbic acid). An example is the binding of boric acid, $B(OH)_3$ or more accurately of borate ions, to the *cis*-hydroxyls (at positions C-2 and C-3) of two β-D-apiose residues (**6-39**) located in different chains of a structural type of pectin called rhamnogalacturonan II, which are cross-linked in this way (see Section 4.5.6.6.1).



6-39, boric acid complex with β-D-apiofuranose in pectin

Boron is an essential element for plants. Whether it is essential for animals has not yet been unequivocally confirmed. Nevertheless, the biological effects of some compounds of boron are known. Boron has an impact on the effect of parathormone (also known as PTH or parathyrin), a hormone secreted by parathyroid glands, which increases blood levels of calcium by its mobilisation from bones (the antagonist of parathormone is calcitonin) and

affects the metabolism of magnesium, calcium, phosphorus and cholecalciferol. Boric acid also affects the activity of many enzymes, such as chymotrypsin, pyridine nucleotide-disulfide oxidoreductase and flavin oxidoreductase.

The recommended daily intake of boron is <20 mg. Actual amounts are dependent on food composition and local conditions. It is estimated that people ingest about 2–10 mg of boron a day, but boron deficiency in humans has not yet been recorded. Boron contained in foods is easily absorbed in the gastrointestinal tract, but 30–92% of boron ingested is excreted in the urine. At higher doses of boric acid, boron accumulates in the nervous system.

An overview of the boron content in some foods is given in Table 6.12. Foods of animal origin are very poor sources of boron. The boron content of meat, fish, eggs and dairy products does not usually exceed 0.3 mg/kg. The highest amounts of boron contain plant products such as legumes, nuts (tens of mg/kg) and fruits (units of mg/kg). The content of boron in vegetables and cereals is usually <2 mg/kg. Wines have a relatively high content of boron (2–11 mg/l). The plant content of boron is dependent on its level in the soil. In addition to the data listed in Table 6.12, significantly higher concentrations of boron in fruits and vegetables have also been found (apples 468, tomatoes 1258 mg/kg dry matter). Boric acid and disodium tetraborate are allowed to be used for the preservation of certain foods (such as caviar) in some countries.

6.3.19 Silicon

Silicon is a metalloid used as a structural material referrd to as biogenic silica (polymerised silica acid) by the major group of algae known as diatoms, by amoeboid protozoa (radiolarian) and also by siliceous sponges. Some plants can deposit silicon within different intracellular and extracellular structures, and silica from decayed plants is re-deposited in the soil in the form of microscopic structures known as phytoliths. Some grasses and horsetails (plants of the genus *Equisetum*, Equisetaceae) contain up to 20% of silicon dioxide (SiO_2). Although silicon was assumed to be an ultra trace element, its exact function in animals is still under discussion. It is assumed that silicon is essential for the synthesis of collagen and connective tissue integrity. The silicon is probably in the form of *ortho*-silicic acid (H_4SiO_4) and disilicic acid ($H_2Si_2O_5$), bound by ester bonds in some mucopolysaccharides. The total silicon content of the human body is about 1.4 g.

The mechanism of silicon resorption in the digestive tract is not known. It is assumed that the available forms of silicon (contained in the diet) are *ortho*-silicic acid and soluble silicates. When given in the form of silicon aluminosilicates, only about 1% of silicon is resorbed. In contrast, some organosilicon compounds used in medicine (such as methylsilantriol salicylate) are resorbed of up to 70%. Resorption efficiency of silicon contained in the diet varies over a wide interval of 5–100%. Beer has a high bioavailability (about 50%) of silicon. The efficiencies of silicon resorption from meat, dairy and soy products usually exceed 50%. From cereals and other foods containing high amounts of silicon, this element is resorbed poorly. Silicon is excreted mainly in the urine.

The silicon content in certain foods is given in Table 6.12. Most foods of animal origin (milk, the meat of mammals and birds) contain only traces of silicon, but higher silicon content has been detected in marine molluscs. In contrast, cereals, mainly barley and oats, have high silicon contents. Wheat contains less silicon than barley. During brewing, most of the grain silicon remains in the husk, but significant quantities of silicon (bioavailable *ortho*-silicic acid) are extracted into the wort and much of this gets into the beer, which is relatively rich in silica (10–30 mg/l). A large share of the silicon is also contained in the surface layers of rice. Brown rice contains 110–570 mg/kg, while in peeled (polished) rice the silicon content is 30–90 mg/kg. Examples of fruits and vegetables with higher silicon contents are bananas and spinach.

The recommended daily dose of silicon has not yet been established. The total daily intake of silicon in the diet is estimated to be 20–50 mg. Silicon intake (e.g. in beer) can reportedly reduce the bioavailability of aluminium, which has been implicated as one of the possible causal factors contributing to Alzheimer's disease. Silicone intake might also help to prevent bone thinning and osteoporosis. High doses of silicon may contribute to the formation of kidney and urinary stones.

6.4 Non-essential elements

6.4.1 Aluminium

Aluminium has long been considered a non-toxic and non-essential element. Today, however, some of its adverse health effects are known. The content of aluminium in the Earth's crust is 8% and aluminium is the third most abundant element (after oxygen and silicon). The content of aluminium in biological materials is low to trace (e.g. 10–50 mg/kg dry matter in grass, 100–300 mg/kg dry matter in leaves of trees and 0.2–0.6 mg/kg in animal tissues), except the lungs that contain aluminium at a level of 20–60 mg/kg.

Aluminium compounds contained in rocks, soil and sediments are very slightly soluble, and in natural waters very low concentrations of aluminium occur (mostly <20 μg/l). Aluminium ions are easily hydrolysed even in neutral media and form insoluble multinuclear complexes of either aluminium ions or aluminium hydroxide $Al(OH)_3$. In addition, in a soil solution at neutral pH, the aluminium concentration is low and its intake by plants is very limited. For example, soil acidification by acid rain increases the solubility of aluminium compounds and aluminium can thus enter the hydrosphere and biosphere in higher amounts. In slightly acidic solutions (pH 5), the predominant cations are $[Al(OH)_2]^+ > [Al(H_2O)_6]^{3+} > [Al(OH)]^{2+}$. Under these conditions, aluminium is actually toxic to certain organisms. A higher intake of aluminium by birds leads to abnormalities of their eggs. Soluble compounds of aluminium in plants cause slower growth, because aluminium is a phytotoxic substance.

6.4.1.1 Occurrence in foods and dietary intake

Older data on the content of aluminium in foods are often overestimated, since the determination of trace amounts of aluminium

(<0.1 mg/kg) in biological materials is very difficult and the published data should be assessed critically.

Aluminium is found in larger amounts in foods of plant origin than in foods of animal origin. Tealeaves have very high concentrations of aluminium (850–1350 mg/kg, and older leaves can even have up to 5000 mg/kg dry matter), because the tea plants (*Camellia sinensis*, Theaceae) accumulate aluminium from the soil. Aluminium present in tea leaves is soluble in hot water (about one-third), so that the brine prepared from 1 g of tea and 100 ml of water has an aluminium concentration 2.7–4.9 mg/l. Aluminium in tea is mainly bound in the form of complexes with catechins, less often with organic acids (such as chlorogenic, gallic, citric, malic and oxalic acids) and fluorine. Complexes with fluorine have the general composition $AlF_n^{(3-n)+}$. The plant obtains them from the soil mainly as AlF^{2+} and AlF_2^{+} cations. Higher aluminium contents are also found in particular spices, such as basil (310 mg/kg), bay leaf (440 mg/kg), oregano (600 mg/kg), pepper (140 mg/kg), cinnamon (80 mg/kg) and thyme (750 mg/kg).

Cereals have moderate amounts of aluminium (e.g. the aluminium content is 4–14 mg/kg in wheat flour, 3–6 mg/kg in wheat bread, 5–6 mg/kg in peeled rice, 5–64 mg/kg in unpeeled rice and 2 mg/kg in maize) and legumes (lentils 15–22 mg/kg, peas 14–27 mg/kg), a lower aluminium content is found in some root crops, vegetables (potatoes 1–6 mg/kg, carrots 1–6 mg/kg, spinach 5–25 mg/kg, cauliflower 0.2–12 mg/kg), meat (pork 2–5 mg/kg, beef 0.2–5 mg/kg) and fish (0.4–9 mg/kg). As already mentioned, recent results have quoted lower concentrations of aluminium in vegetables (0.1 mg/kg in cabbage, 0.05 mg/kg in carrots and 0.2 mg/kg in potatoes). Offal sometimes contains medium to high amounts of aluminium (e.g. beef liver 4–46 mg/kg, pork kidney 2–18 mg/kg and pork brains 7–130 mg/kg). The concentrations of aluminium in milk, dairy products and eggs are very low (0.011–0.035 mg/kg in milk, 0.15–0.34 mg/kg in Edam cheese, <0.2 mg/kg in eggs). The aluminium concentration in breast milk generally ranges from 0.004 to 0.03 mg/kg, but can also reach values of from 0.2 to 2.4 mg/kg.

Some aluminium compounds, such as sodium aluminium phosphate, $Na_8Al_2(OH)_2(PO_4)_4$, are used in the United States and other countries as additives (e.g. as a baking powder components). The aluminium content in foods can also be increased during their contact with metal aluminium. Some foods and beverages are commonly packed in aluminium foils, tubes or cans. Until recently, aluminium barrels were used for beer and soft drinks. Also, during the cooking of foods in aluminium cookware, the aluminium can be partially dissolved. This applies especially to acidic foods and drinks, such as fruits, fruit puree and juices, and vegetables in vinegar brine.

The average daily dietary dose of aluminium for adults, found in Western countries, ranges from 4 mg (Switzerland 1985) to 27 mg (United States 1985). Sources other than foods, such as some medicinal drugs, may sometimes be more important. For example, oral antacids neutralising gastric juices contain aluminium oxide or aluminium hydroxide. Daily dietary doses of aluminium compounds can then reach 2 g. Aluminium is also contained in various toothpastes and cosmetic preparations.

6.4.1.2 Metabolism and toxic effects

The effectiveness of aluminium resorption in the gastrointestinal tract of humans under normal conditions is about 0.1–0.3%. Lactic, citric and ascorbic acids facilitate gastrointestinal resorption. Approximately 95% of orally administrated aluminium binds to transferrin and albumin and then is excreted in the urine. The normal concentration of aluminium in blood serum is 2–40 μg/l, and the concentration of aluminium in various organs, except the lungs, is very low and does not increase with age. The total aluminium content in the human body is about 35 mg.

Aluminium has the potential to accumulate, especially when the gastrointestinal barrier is bypassed. If a significant load exceeds the excretory capacity of the body, the excess is deposited in various tissues, including bones, brain, liver, heart, spleen and muscles. It is now commonly acknowledged that aluminium toxicity can be induced by oral exposure (as a result of aluminium-containing pharmaceutical products such as aluminium-based phosphate binders or antacid intake) and by infusion of aluminium-contaminated dialysis fluids. In the early 1970s, aluminium toxicity was first implicated in patients with chronic renal failure involving bone (renal osteomalacia) or brain tissue (dialysis encephalopathy) who were undergoing dialysis. For example, with intravenously infused aluminium, 40% is retained in adults and up to 75% is retained in neonates. The exact mechanism of aluminium toxicity is not completely understood. It is known that aluminium combines with many proteins and cofactors that are required in intermediary steps of metabolism. There is probably a link between aluminium intake and increased incidence of Alzheimer's disease.

6.4.2 Tin

The average tin content of the Earth's crust is 3 mg/kg. In nature, tin occurs mainly as the mineral cassiterite (stannic oxide, SnO_2) and as an accompanying metal in some sulfides. Metallic tin is an important component of conventional alloys (bronze) and of cans made of tinplate (tin-coated steel) and is used in the food industry. A large amount of tin is used for the production of organometallic compounds (annual production of about 30 000 tons). Dibutyltin dilaurate and analogous octyltin compounds are used as stabilisers of plastics, i.e. poly(vinylchloride), PVC. Tributyltin compounds are used for wood preservation and as ingredients in special paints for ships and other bodies exposed to the long-term effects of seawater. Triphenyltin acetate and triphenyltin hydroxide are used in agriculture as fungicides.

Natural fresh waters contain only trace amounts of tin (0.01–1 μg/l), with concentrations of about 3 μg/l in sea water. Aquatic organisms can accumulate tin (bioconcentration factor is 10^2–10^3). Some microorganisms may methylate inorganic compounds and dealkylate synthetic organometallic compounds. Tin concentrations in soils are in the range of 2–200 mg/kg dry matter.

The natural content of tin in foods is very low and mean concentrations are <1 mg/kg in most foods (meat 0.007, meat products 0.18, offal 0.014, poultry 0.006, fish 0.032, milk 0.003, dairy products 0.297, eggs 0.003, fresh fruit 0.019, green vegetables 0.003,

other vegetables 0.05, potatoes 0.004 and nuts 0.029 mg/kg fresh weight). High levels of tin are often a result of migration from cans. Although tin is corrosion-resistant, acidic foods such as fruits and vegetables can still cause corrosion of the tin layer. Hence, fruit juices and canned vegetables can contain a significantly higher level of tin (e.g. 30–260 and 41 mg/kg, respectively). A 2002 study showed that 99.5% of 1200 tested cans contained less than the UK regulatory limit of 200 mg/kg of tin. In recent years, the use of tinplated cans for fruit juices, canned fruit and other acidic foods has been restricted. In some foods, traces of tin organometallic compounds may be found, for example wines transported in PVC containers contain dibutyl tin at concentrations of up to 160 μg/l.

The daily dietary intake of tin is estimated at 3 mg. The tolerable daily intake for an adult man (weighing 70 kg) is 140 mg. The effectiveness of resorption of tin in the gastrointestinal tract depends on the metal valence (stannous compounds 3–8%, stannic compounds about 1%). The normal concentration of tin in blood plasma is 30–40 μg/l. The tin that is absorbed is excreted in the urine and partly in the bile. Nausea, vomiting and diarrhoea have been reported after ingesting canned food containing 200 mg/kg of tin. Toxic effects of tin appear in cases of long-term consumption of foods with a high content of tin (1400 mg/kg). Organometallic compounds of tin are highly toxic.

6.5 Toxic elements

The most important toxic elements include lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As). We may also include many trace elements (and the radioactive metals) when considering high toxic doses. Examples of such metals are iron, zinc, copper, manganese, molybdenum, cobalt and chromium, which are required by living organisms. The term **heavy metals** is often incorrectly used as a synonym of toxic elements to describe the group of toxic metals, but lighter metals such as beryllium (Be) also show toxicity, arsenic is a metalloid, and not all heavy metals are particularly toxic. Strictly speaking, the term heavy metals should only be used for those elements with an atomic weight of 200 and above, such as mercury (200), thallium (204) and bismuth (209).

Excessive levels of toxic elements can be damaging to the organism as they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in an organism over time, compared with its concentration in the environment. The presence of toxic chemical elements in foods is mainly connected with environmental pollution. A number of anthropogenic and natural sources contribute to the toxic elements entering into the food chain. The main anthropogenic causes of contamination by toxic elements are burning fossil fuels, transport, industrial production of metals, use of various elements in industry and technology and their related waste, excessive use of mineral fertilisers and other agrochemicals (such as pesticides) or the application of sewage sludge on to the soil. Natural sources of toxic elements in the environment include the weathering of rocks, forest fires and volcanic activity.

There are some sources that contribute substantially to the contamination of one particular element (e.g. lead contamination of the atmosphere from the exhaust of motor vehicles that use leaded gasoline), but most causes cannot be characterised in this way. For example, the environmental contamination in the vicinity of metallurgical plants is manifested by significantly elevated levels of many elements (Cd, Pb, Tl, In, As, Ga, Hg, Ni, Cr, Cu, Zn and Ge), simultaneously in different sections of the environment. This is due to the polymetallic character of the processed ore, generated intermediate products and waste. Emissions from thermal power plants and other energy sources, including incinerators, contain many metals simultaneously. It is therefore quite difficult to identify specific sources of contamination of individual elements. In addition, air and water flows act as transmission media of toxic elements in the environment. In particular, there can be metals that occur in solid particles and aerosols or in the gas phase (mercury and organometallic compounds; arsine, H_3As; various methylarsins) transmitted in the atmosphere over large distances. The fallout of solid particles and wet deposition are the main routes of surface contamination of soil and plants. Wet deposition occurs when rain or snow removes metals from the atmosphere and delivers them to the ground, plants or other surfaces.

The level of toxic elements in foods is among the main indicators of pollution, ecological risk and food safety assessment. For lead, cadmium, mercury and arsenic (as well as for tin, aluminium, chromium, copper, nickel, zinc and iron), the tolerable daily intake levels of individual minerals that are unlikely to pose a risk of adverse health effects have been established for raw and finished food products, and specifically for individual groups of foods in many countries. The levels of aluminium and iron are limited only for selected commodities. Examples of maximum levels for mineral elements in foods are summarised in Table 6.13. At the European level, the maximum levels are given only for lead, cadmium, mercury and tin. The maximum levels for lead and cadmium are given for various types of food of plant and animal origin. Mercury is limited only in the flesh of fish, such as tuna, shark and other seafood. Generally, this limit is set at 0.5 mg/kg (wet weight) and for listed species at a level of 1.0 mg/kg. Recently, it was shown that food supplements (especially if they contain seaweed, which naturally accumulates cadmium) can contribute significantly to human exposure to lead, cadmium and mercury. Therefore, limits for these products have been included. Tin is limited in all canned foods other than beverages (200 mg/kg) and canned beverages, including fruit juices and vegetable juices (100 mg/kg). The maximum levels for canned infant formulae, baby foods and foods for young children are only 50 mg/kg. For some foods, which are not subject to limits for lead, cadmium, mercury and tin in European legislation (Table 6.13), there are national limits for these and some other elements (such as As, Cu, Fe and Zn) that are still valid.

Heavy metal poisoning is treated by substances with a complexing effect, which immobilise the metal ions prior to excretion. Examples of these chelating agents are 2,3-dimercaptopropan-1-ol (known as Dimercaprol or British Anti-Lewisite, abbreviated BAL), 2,3-dimercaptosuccinic acid, 2,3-dimercaptopropane-1-sulfonic acid

Table 6.13 Examples of maximum levels for mineral elements in foods.[a]

Food	Maximum levels (mg/kg fresh weight)						
	Cd	Pb	Hg	As	Cu	Fe	Zn
Meat	0.05	0.1	0.05	-	-	-	-
Fish, seafood, liver, kidney	0.05–1.0	0.3	0.5–1.0	-	-	-	-
Milk	0.01	0.02	-	-	-	-	-
Eggs	0.02	-	-	-	-	-	-
Cereals, rice, bakery products	0.1–0.2	0.2	0.03–0.05	-	-	-	-
Fruits, vegetables, potatoes	0.05–0.2	0.1–0.3	0.02–0.03	0.2	-	-	-
Mushrooms	0.2–1.0	-	-	-	-	-	-
Fruit drinks	0.05	0.05	-	0.2	-	-	5.0
Fats, oils, margarine	-	0.1	-	-	0.1–0.4	1.5–5.0	-
Child and infant nutrition	0.1	-	0.02	0.1			
Food supplements	1.0–3.0	3	0.1	-	-	-	-

[a]Official Journal of the European Union, 20.12.2006, L 364/5: Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 3.7.2008, L 173/6: Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuffs.

(abbreviated DMSA; the meso isomer and its sodium salt, known as Unithiol), glutathione, ethylenediaminetetraacetic acid (abbreviated as EDTA) and its salts. Dimercaprol is used in the treatment of arsenic, lead, mercury and other toxic metal poisoning, DMSA for treating lead and mercury poisoning, EDTA for treating lead and mercury poisoning and to remove excess iron from the body. For treatment of arsenic poisoning, similar or identical substances, such as those for mercury poisoning (2,3-dimercaptopropan-1-ol, 2,3-dimercaptosuccinic acid, glutathione, selenium preparations) are used.

6.5.1 Lead and cadmium

The average lead content in the Earth's crust is 13 mg/kg and cadmium content is 0.1 mg/kg. In nature, these metals are found mainly in sulfide and carbonate ores. Lead sulfide is known as galena (PbS), cadmium sulfide occurs in the rare ore greenockit (CdS), and both metals are present as admixtures in sphalerite (ZnS) and smithonite ($ZnCO_3$).

Metallic lead is used to make batteries, sheets and pipes (including water pipes). Inorganic lead compounds are found in various pigments (such as orange lead tetroxide, also called minium, Pb_3O_4, and yellow lead chromate, $PbCrO_4$, or are used in the production of lead glass (lead oxide, PbO). Organometallic compounds of lead, tetraethyl and tetramethyl lead, were used in gasoline as antiknock additives. Every year, the world production of lead amounts to about 5 million tons.

Cadmium is used in anticorrosive metal protection coatings and in battery production. Cadmium sulfide is used as a yellow pigment, and cadmium salts of fatty acids as stabilisers in PVC production. The annual world production of cadmium is about 20 000 tons.

6.5.1.1 Occurrence in environment, transport and distribution

The levels of lead and cadmium in the air vary locally. In a few areas, polluted air contains 0.005–0.3 μg/m^3 of lead and 0.0001–0.002 μg/m^3 of cadmium. In contrast, the air in large cities has been found to contain 0.2–5 μg/m^3 of lead and 0.007–0.05 μg/m^3 of cadmium. Natural waters contain only traces of lead and cadmium. Lead concentrations in uncontaminated river and lake waters range from 0.1 to 5 μg/l and the cadmium content is even lower (0.07 to 0.8 μg/l). Significantly higher concentrations of these metals are found in sediments of uncontaminated rivers and reservoirs (10–30 mg/kg of lead, 0.04–0.8 mg/kg of cadmium). The contents in sediments from contaminated localities can be up to several orders of magnitude higher. Numerous aquatic organisms (algae and other aquatic plants, zooplankton, crustaceans and molluscs) accumulate cadmium and other elements (mercury, arsenic and selenium) in their bodies to a significant exent. Even if they live, such as oysters, mussels or shrimp, in only slightly contaminated water (concentration of cadmium in water is in units of μg/l), the level of cadmium in their bodies can reach up to 100 mg/kg. The content of lead in uncontaminated soil can range from 5 to 40 mg/kg dry matter, and cadmium in the range of 0.2–1 mg/kg dry matter (the concentrations in polluted localities may be much higher).

In addition to the level of toxic elements in the soil, their availability to plants is another important factor. Plants take toxic elements either from the soil through their root system or from the atmosphere through deposits mainly on the surface of their leaves (foliar deposits). The intake of toxic elements through the root system depends not only on the type of plant and the content of relevant elements in the soil, but also on the distribution

of metals in the soil horizon depths, the metal mobility in the soil, organic matter content and other factors. The bioavailability of heavy metals for the plant strongly depends on the pH and redox potential of the soil solution. The relative mobility of lead and cadmium (and mercury) increases in acidic and oxidising environments. Transition coefficients of elements for soil–plant transfer reach values ranging from 0.01 to 0.1 for lead, and from 0.1 to 10 for cadmium. Intake of individual elements by plants is quite different between plant species and even between different cultivars. Some plants accumulate only certain elements. In these cases, the element does not possess strong phytotoxic effects. Plants that accumulate cadmium and lead from the soil include spinach, lettuce and some oilseeds. Cereals accumulate only small amounts of these elements.

Distribution of metals in different parts of the plants is not uniform. If foliar versus root intake is negligible (less polluted localities), the concentrations of these elements generally decrease in the order: roots > leaves > stems > fruits > seeds. These factors should be taken into account when assessing the levels of toxic elements in plants, because only certain parts of the same plant are consumed by herbivores, and only certain parts of cultivated plants are processed for food or feed use. It is in this way that the toxic elements present in the plants enter the food chain.

Contamination of the environment by the toxic elements increases their content in the organs and tissues of animals. Toxic elements enter the bodies of animals in particular by the oral route via consumed food. In human nutrition, the concentration of these elements in muscle and viscera of animals (farm as well as wild animals) is the most important factor. The content of lead and cadmium in the liver and kidneys of wild animals indicates the load of the contaminated animal food and habitat pollution level in which an animal lives. Under normal conditions, the content of lead and cadmium in meat is very low (thousandths of mg/kg), in contrast to the concentration in the kidney and liver, which are sometimes up to 2–3 orders of magnitude higher. In these organs, lead and cadmium accumulate significantly.

6.5.1.2 Occurrence in foods and dietary intake

The content of lead and cadmium in foods is low and very variable. A survey of toxic element levels in major foods and food raw materials is given in Table 6.14. For plant foods, the concentrations of lead and cadmium depend mainly on the content of these elements in the soil. Relatively high concentrations (in hundredths to tenths of mg/kg) are characteristic of some vegetables (spinach, lettuce and carrots), edible mushrooms and oil seeds (e.g. poppy seeds contain 0.04–1.96 mg/kg of cadmium). Although grains have fairly high maximum values, in most cases cadmium concentrations do not exceed 0.1 mg/kg and lead concentrations 0.3 mg/kg. Relatively high concentrations of lead were found in wines (0.016–0.17 mg/l). The highest content of lead and cadmium from foods of animal origin is in offal, the internal organs and entrails of butchered animals. The level of cadmium in the kidney is especially high, and can reach up to units of mg/kg. The toxic metal content in the liver and kidneys of animals is related to diet and age of the animals. In older animals, concentrations are higher. Meat, eggs, milk and dairy products contain only traces of lead and cadmium. Higher levels of lead are often found in foods packed in cans. This is due to contamination with lead contained in the alloy of tin, which is found in the seam of sealed cans. Packing of foods in glass rather than cans is recommended for foods that require more stringent standards (such as baby food).

The tolerable daily intake of lead is 250 μg and of cadmium 70 μg (at 70 kg body weight). The actual daily dietary dose of lead found in several studies in European countries formerly ranged from 27 (Sweden 1983) to 180 μg (Belgium 1983). Increased doses of lead and consequently higher levels of lead in blood were observed in wine drinkers. The long-term monitoring of the actual average daily dietary doses from the 1970s to late 1990s recorded a significant drop in the lead intake (e.g. in Great Britain the values dropped from about 110 to about 25 μg). Actual daily dietary intake of cadmium in the 1980s ranged from 10 (Sweden 1983) to 33 μg (United States 1985). Long-term studies also found some decrease in daily dietary intake, but this was less pronounced in comparison with lead.

6.5.1.3 Metabolism and toxic effects

Lead and cadmium enter the body not only in food through the digestive tract, but also through the lungs. For smokers, the proportion of inhalation exposure to cadmium intake is comparable to the intake of this element from food. The level of cadmium in tobacco is about 1–2 mg/kg. Resorption of lead depends on age, composition of the diet and health. The effectiveness of resorption in adults is estimated at 10%. A child's body resorbs around 40–50% of lead from food. Lead resorption is higher in foods containing higher amounts of protein, and lower in the presence of large amounts of fibre, phytic acid, iron and calcium. The normal levels of lead in blood are in the range of 50–120 μg/l. The proportion of resorbed cadmium amounts to an average of 6% or more (e.g. the amount from oatmeal is 4–37%). Blood levels of cadmium in non-smokers are 0.2–3 μg/l, and in smokers 0.2–5 μg/l.

Resorbed lead and cadmium are transported by blood to the liver and kidneys, where these elements accumulate. Part of the lead in the liver is excreted via the bile into the intestine. A small percentage of lead is excreted in the urine. Long-term exposure leads to lead accumulation in bones. During intoxication, lead or cadmium can damage the kidneys and liver. Lead also damages the blood, as well as the nervous and cardiovascular systems. Cadmium has teratogenic and carcinogenic effects, damages reproductive organs and affects blood pressure. Children are particularly susceptible to lead poisoning (due to the possibility of ingestion of soil or dust, higher resorbed amounts and higher sensitivity of the organism). Even at a level of 150 μg/l in blood, a number of adverse effects may appear (such as slower mental and physical development, lower learning ability, lower intelligence and decreased immunity).

Lead inhibits the synthesis of porphyrins, so that in chronic poisoning the amount of haemoglobin in erythrocytes decreases and anaemia develops. Lead is an inhibitor of two enzymes essential for the synthesis of haem, δ-aminolevulinic acid dehydratase and ferrochelatase. Aminolevulinic acid dehydratase catalyses condensation of two molecules of δ-aminolevulinic acid to form

Table 6.14 Lead, cadmium, mercury and arsenic contents in important food raw materials and foods.[a]

Food	Content (mg/kg)			
	Pb	Cd	Hg	As
Pork meat	0.005–0.05	0.001–0.01	0.002–0.006	0.003–0.03
Beef meat	0.004–0.07	<0.001–0.01	0.001–0.003	0.001–0.07
Chicken meat	0.008–0.04	0.001–0.005	0.001–0.002	0.001–0.03
Pork liver	0.014–0.04	0.025–0.10	0.007–0.014	0.005–0.02
Beef liver	0.01–0.42	0.03–0.17	0.001–0.005	0.005–0.07
Pork kidney	0.01–0.04	0.07–0.52	0.011–0.015	0.01
Beef kidney	0.06–0.22	0.06–2.0	0.003–0.014	0.02–0.13
Sea fish	0.01–0.14	0.001–0.07	0.03–0.85	0.50–1.4
Sweetwater fish	0.01–0.05	0.001–0.005	0.07–1.01	0.03–0.56
Milk (whole)[b]	0.001–0.002	<0.0001–0.001	<0.001	<0.001–0.003
Curd	0.02	<0.002	<0.001	0.01
Cheese	0.01–0.06	0.005–0.02	<0.002	<0.002–0.025
Yoghurt	0.01–0.03	0.001–0.003	<0.001	<0.005
Eggs	0.001–0.01	0.001–0.01	0.005–0.008	<0.002–0.01
Wheat	0.02–0.65	0.02–0.35	0.0001–0.006	0.005–0.29
Flour (wheat)	0.004–0.05	0.01–0.09	0.002–0.004	0.01–0.17
Bread (whole wheat)	0.012–0.013	0.02–0.05	0.001–0.006	0.006–0.05
Rice (peeled)	0.003–0.08	0.004–0.14	0.002–0.008	0.04–0.31
Rye	0.01–0.17	0.004–0.04	0.002–0.007	0.03–0.10
Barley	0.03–0.27	0.004–0.04	0.001–0.006	0.005–0.38
Oats	0.03–0.30	0.004–0.07	0.0001–0.008	0.01–0.54
Peas (mature seeds)	0.01–0.43	0.01–0.03	0.002–0.02	0.01–0.05
Beans	0.02–0.10	0.003–0.02	0.004–0.02	<0.01
Soybeans	<0.002–0.32	0.04–0.09	<0.004	0.03–0.05
Cabbage	0.002–0.04	0.01–0.017	0.0003–0.001	<0.01
Cauliflower	0.002–0.02	0.002–0.02	0.0004–0.002	0.002–0.01
Spinach	0.01–0.29	0.01–0.35	<0.001–0.008	0.005–0.02
Lettuce	0.003–0.25	0.002–0.16	0.0005–0.01	0.002–0.14
Tomatoes	<0.001–0.04	0.002–0.05	0.0001–0.008	<0.001–0.002
Carrot	0.004–0.21	0.003–0.16	0.0006–0.005	0.003–0.11
Peas (green)	0.01–0.02	0.001–0.03	0.0005–0.002	0.01
Onion	<0.001–0.05	0.004–0.05	<0.001	0.01
Potatoes	0.006–0.04	0.002–0.06	0.0001–0.017	<0.001–0.04
Mushrooms	0.01–0.20	0.01–0.33	0.07–0.22	0.01
Apples	0.01–0.05	0.001–0.002	0.0003–0.002	0.001–0.22
Oranges	0.005–0.07	0.001–0.007	<0.001	0.004–0.02
Bananas	0.02–0.05	<0.002	0.001–0.002	0.04–0.09

(*continued overleaf*)

Table 6.14 (*continued*)

Food	Content (mg/kg)			
	Pb	Cd	Hg	As
Strawberries	0.006–0.09	0.001–0.03	0.0002–0.001	<0.005
Grapes	0.012–0.024	0.001–0.002	0.0004–0.002	0.01–0.16
Peanuts	0.01–0.19	0.01–0.51	<0.004	-
Tea (black)	0.07–1.29	0.005–0.12	0.007–0.025	0.05–0.40
Coffee (roasted)	0.02–0.05	0.003–0.007	<0.004	0.05–0.22
Cocoa	0.03–0.07	0.095–0.17	<0.004	0.10
Chocolate (milk)	0.05	0.005–0.01	0.002–0.004	<0.05

[a] Table summarises the data from Sweden, Finland, Holland, Germany, Canada and the United States.

[b] The breast milk concentrations are: Pb 0.0004–0.002, Cd 0.0001–0.0006, Hg 0.0003–0.001 and As <0.001 mg/kg.

porphobilinogen. Ferrochelatase catalyses the incorporation of iron into protoporphyrin IX to form haem. Bodily exposure to lead can also damage the central and peripheral nervous system. In some cases, lead poisoning leads to motor skills disorders and slowing of movement reactions, and to similar symptoms. Neurotoxic effects of lead are particularly dangerous for children. Brain damage (encephalopathy) occurs during heavy exposure to lead (blood levels of 800–1000 μg/l).

Cadmium poisoning can cause acute kidney failure. The kidneys are the organ that accumulates the most cadmium (in adults the kidney contains 4–10 mg of Cd). The normal concentration in the renal cortex of humans is 10–30 mg/kg. Formation of metallothionein prevents acute renal damage, if the concentration of cadmium in the renal cortex does not exceed about 100 mg/kg. Intoxication with cadmium is manifested by the occurrence of proteins and sugars in the urine, but only very small amounts of cadmium are excreted in the urine. Cadmium also accumulates significantly in the liver (normally in the amount of 2–4 mg). At concentrations not exceeding 30 mg/kg of tissue, the binding of cadmium to liver metallothionein prevents liver damage. Cadmium poisoning also results in decalcification and softening of bones. Itai-itai disease was the first well-documented case of mass cadmium poisoning by rice (containing 1–3 mg/kg of cadmium) that was irrigated with contaminated river water in Toyama Prefecture, Japan, officially recognised in 1968.

6.5.2 Mercury

The average mercury content in the Earth's crust is about 0.05 mg/kg. In nature, mercury occurs in deposits mostly as mercuric sulfide, cinnabar (HgS). Roasting of the sulfide ore produces carbon dioxide and elemental mercury vapour, which condenses to give liquid mercury. The annual global production of mercury is about 7000 tons. Owing to its high toxicity, limits on the use of mercury have been introduced. Mercury is used mainly:

- in electrical engineering for the production of batteries, switches, electrodes and measuring instruments;
- for the electrochemical production of chlorine and caustic soda by electrolysis of NaCl solution (mercury is the cathode);
- for the manufacture of paints (a red pigment, vermilion, is mainly obtained by reduction from cinnabar), catalysts and fungicides (phenylmercuric chloride for seed treatment);
- for preparation of dental amalgam used as a filling material in dentistry.

6.5.2.1 Occurrence in environment, transport and distribution

The input of mercury into the environment is mainly caused by volcanic activities, burning coal, using mercury in industry and agriculture and through handling of waste. The total amount of mercury entering the atmosphere is estimated at 150 000 tons per year. About two-thirds of this amount comes from natural resources.

Migration of mercury in the environment depends on some of the important properties of mercury and mercury compounds. Organometallic compounds of mercury and elemental mercury are volatile and fat soluble (solubility of metallic mercury in fat is approximately 5–50 mg/l). At a temperature of 24 °C, an atmosphere saturated with mercury contains 18 mg Hg per m^3. Mercuric salts are mostly water soluble. Mercurous chloride (Hg_2Cl_2) has a very low solubility (2 mg/l), mercuric chloride is more soluble (74 g/l at 20 °C) and mercuric sulfide (HgS) is practically insoluble.

The level of mercury in the air of large cities is in the tens to units of ng/m^3. Natural waters contain only trace amounts of mercury. The concentration of mercury in rivers is in the region of 0.01–0.2 μg/l (while the permissible limit for drinking water

is 1 μg/l). The concentration of mercury in seawater is even lower (0.0004–0.002 μg/l). Despite their very low concentrations, the oceans and seas represent a large reservoir of mercury. For example, the Mediterranean Sea contains about 3700 tons of mercury, and each year through fallout and tributaries some 240 tons enter it, with about 80 tons per year going into the marine sediment and 150 tons of mercury evaporating into the atmosphere.

In the aquatic environment important chemical transformations of mercury species also take place, in particular the methylation reaction of bacteria and microscopic fungi, oxidation–reduction reactions and precipitation reactions. The products of mercury biomethylation are the organometallic compounds methylmercury and dimethylmercury. In anaerobic conditions (as in the depths of the seas), mercuric salts can be reduced to elemental mercury or mercuric ions, and little soluble Hg_2Cl_2 is formed. Mercury compounds are susceptible to disproportionation. In the formation of slightly soluble compounds (mainly HgS), mercury is transferred from water to the sediment. The concentration of mercury in non-polluted sediments of rivers and lakes ranges from the tenths to units of mg/kg dry matter. About 1% of mercury in sediments is present in the form of methylated compounds. Aquatic organisms accumulate mercury from the water. The highest bioconcentration factors (see Section 12.1) were found in invertebrates (1×10^5), with lower factors in freshwater fish (6×10^4) and marine fish (1×10^4). The concentration of mercury in their bodies is several orders of magnitude higher than in the surrounding environment. About 90% of mercury in fish is methylated.

The concentration of mercury in uncontaminated soils ranges from 0.02 to 0.2 mg/kg. The main forms of mercury in neutral and alkaline soils are the slightly soluble oxide (HgO) and carbonate ($HgCO_3$). Sulfur bacteria produce almost insoluble sulfide (HgS). Most of the remaining forms of mercury are adsorbed on organic matter, hydrated iron and manganese oxides and clay minerals. Owing to the low mobility of mercury in the soil, only small amounts of mercury pass from the soil to plants.

The mercury content of plants is in the tenths to tens of μg/kg. Some mushrooms contain higher concentrations of mercury (tenths to units of mg/kg). The level in the bodies of animals is dependent on the composition of their food. High concentrations of mercury were found, for example, in the liver and kidneys of aquatic birds. Environmental pollution with mercury and other metals is often monitored using bioaccumulative indicators (biomonitors) such as the fur of mammals or the feathers of birds.

6.5.2.2 Occurrence in foods and dietary intake

The concentration of mercury in most foods ranges from ten thousandth to hundredths of mg/kg (Table 6.14). High concentrations of mercury (tenths to units of mg/kg) are found in some edible mushrooms, fish, shellfish and crustaceans. For example, mercury concentrations in fish in the Czech Republic were as follows: carp 0.01 mg/kg, roach 0.08–0.23 mg/kg, tench 0.14–0.64 mg/kg, perch 0.14–2.43 mg/kg, pike 0.16–2.87 mg/kg, zander (pike-perch) 0.34–1.90 mg/kg, eel 0.13–2.40 mg/kg.

The tolerable daily intake of total mercury for an adult is 50 μg, and the tolerable daily intake of methylmercury is 16 μg (at 70 kg body weight). The actual daily dietary dose of mercury found in several studies in Western European countries varied (depending on the composition of the diet) over a very wide range, from 0.7 (Holland 1989) to 14 μg (Belgium 1983). The dietary intake of mercury is critically related to the eating of fish. Owing to the presence of methylmercury, the prevailing form in fish and other sea foods, the tolerable daily intake can very often be achieved almost entirely by consuming these products.

6.5.2.3 Metabolism and toxic effects

About 7% of mercury from food is resorbed in the small intestine. The resorbed mercury is taken up by the liver, kidneys and brain. Part of the mercury is eliminated from the liver via the bile into the intestine. Mercury also accumulates in hair and nails. The toxic effects of mercury and its compounds are related to the high affinity of mercury for thiol groups in peptides and proteins. Binding of mercury to these functional groups occurs in enzymes and leads to their inhibition. The toxicity of various forms of mercury decreases as follows: alkylmercury compounds (mainly CH_3Hg^+) > elemental mercury vapours > mercuric salts (Hg^{II}) > arylmercury (diphenylmercury) and alkoxymercury compounds (RHgOR) > mercurous salts (Hg^I). In terms of food toxicology, methylmercury is the most important compound due to its presence in fish. Water in the Minamata Bay in Japan was heavily polluted in the 1950s and 1960s by wastewater containing methylmercury (units of μg/l) from the Chisso Corporation's factory in Minamata. Methylmercury bioaccumulated in fish and shellfish in the bay, which gave rise to the so called Minamata disease when the fish were eaten, with more than 10 000 people affected. In 1970, Cargill Incorporated (a multinational corporation based in Minnesota, USA) sold seed grain treated with methylmercury to Basra, Iraq. Although this seed was not intended for human or animal consumption, but only for use in agriculture, a number of recipients consumed the surplus seed, which led to the deaths of 93 people.

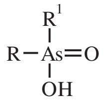
Poisoning by inorganic mercury compounds and by elemental mercury occurs mainly through occupational exposure (workers in chemical plants and laboratories). The main organs that are damaged during intoxication are the kidneys and the brain. Effects of the individual forms of mercury are somewhat different. In methylmercury poisoning, neurotoxic effects prevail, manifesting themselves by disturbances of sensory functions (vision, hearing and balance), speech, swallowing disorders, morphological changes in the brain and mental malfunction. Methylmercury also has teratogenic effects. There is a risk of foetal damage in expectant mothers where the concentration of mercury in the hair reaches about 15–20 mg/kg. The symptoms of poisoning in adults appear when mercury concentrations in hair are above 30 mg/kg. Therefore, it is recommended that pregnant women significantly reduce their consumption of fish.

Poisoning by inorganic mercury compounds can lead to reduced production of urine and even to kidney failure. Poisoning by elemental mercury can occur either by inhalation of mercury vapours or ingestion of mercury. The ingestion of mercury manifests by increased salivation, a metallic taste in the mouth, swollen

gums, loss of appetite, loss of teeth, insomnia, muscle tremors, vomiting, diarrhoea, fatigue, loss of self-control and muscle weakness. The renal function deteriorates and sometimes the thyroid gland is enlarged. Inhalation of mercury vapours leads to some further symptoms (bronchitis, chest pain, coughing and difficult breathing).

6.5.3 Arsenic

The average content of the metalloid arsenic in the Earth's crust is 1.8 mg/kg. In nature, arsenic occurs in the form of sulfide minerals (e.g. arsenopyrite, FeAsS; realgar, As_4S_4; and cobaltite CoAsS) and is included as a minor component in sulfide ores of copper, lead and other metals. The main industrially produced compound of arsenic is arsenic trioxide (As_2O_3). Annual world production of this oxide is about 60 000 tons. Elemental arsenic is used as an ingredient in alloys of lead and other metals. Some inorganic arsenic compounds, such as cupric hydrogen arsenite, $CuHAsO_3$, a green pigment used in the past in some paints (Scheele's Green), copper (II) acetoarsenite, $Cu(CH_3COO)_2{\cdot}3Cu(AsO_2)_2$, known as Paris green, have been used as a rodenticides and insecticides for fruit trees and vines, and also as pigments. Other substances, such as $Pb_3(AsO_4)_2$, $Ca_3(AsO_4)_2$ and Na_3AsO_3, were used for the treatment of tobacco and cotton. Synthetic organic arsenic compounds in the United States and some other countries are used as growth stimulators in pigs and poultry, and as veterinary medicines. These compounds are mainly derivatives of phenylarsonic acid (**6-40**), for example 4-aminophenylarsonic acid (known as arsanilic acid) and 4-hydroxy-3-nitrobenzenearsonic acid (known as roxarsone). Arsanilic acid is used in the prevention and the treatment of swine dysentery in veterinary medicine. Roxarsone was widely used agriculturally as a chicken feed additive to promote growth.



6-40, phenylarsonic acid, R = C_6H_5, R^1 = OH
methylarsonic acid, R = CH_3, R^1 = OH
dimethylarsinic (cacodylic) acid, R = R^1 = CH_3

6.5.3.1 *Occurrence in environment, transport and distribution*

Arsenic enters the environment mainly as a result of smelting activities, the combustion of coal (e.g. fly ashes from thermal power plants in the Czech Republic contain arsenic at levels of 40–110 mg/kg) and from wood preserved by arsenic compounds. In countries where pesticides based on arsenic compounds are permitted, these agrochemicals may be the dominant source. Volcanic activity and weathering of rocks contribute to the entry of arsenic into the environment to a lesser extent. Annual world emissions of arsenic have been estimated at 120 000 tons.

For example, the annual mean concentrations of arsenic in the UK rural environment are typically in the range of 0.001–0.004 $\mu g/m^3$, whilst annual mean urban concentrations are higher, in the range of 0.005–0.007 $\mu g/m^3$. Typically, the highest concentrations are found at sites located in the immediate vicinity of industrial processes such as smelters, incinerators and cement works. The highest reported concentration of arsenic, found in the vicinity of a smelter in Walsall, West Midlands, UK, was 573 $\mu g/m^3$. For the monitoring period covering 1975–1990, the highest annual mean concentration of arsenic reported in Walsall was 0.223 $\mu g/m^3$, whilst for other urban sites, during the same period, annual mean values were found to vary from 0.001 to 0.018 $\mu g/m^3$. The concentrations of arsenic (methylated forms such as methylarsonic and dimethylarsinic acids prevail; **6-40**) in the air are locally variable and are higher in winter (e.g. in Prague, concentrations of 0.56 $\mu g/m^3$ in the winter and only 0.07 $\mu g/m^3$ in summer were found, and the annual average was 0.3 $\mu g/m^3$). In the vicinity of thermal power plants, the concentrations of arsenic are higher (tens of $\mu g/m^3$).

Natural waters, with the exception of some mineral and thermal waters, contain only traces of arsenic. Concentrations in unpolluted river and lake waters are in the range of from 0.15 to 0.45 $\mu g/l$, where the arsenic is found in inorganic compounds. Seawater contains 0.1–2 $\mu g/l$ of arsenic in the form of arsenite (*ortho*-arsenite, AsO_3^{3-}), arsenate (AsO_4^{3-}), methylarsonic and dimethylarsinic acids (**6-40**). Aquatic organisms, particularly marine organisms contain arsenic at concentrations ranging from 1 to 100 mg/kg as a result of bioaccumulation and biotransformation processes. Marine organisms accumulate arsenic compounds from the water and transform them in particular into arsenobetaine (**6-21**), arsenocholine (**6-22**) and other organic compounds. For instance, a marine bivalve mollusc variegated scallop (*Chlamys varia*) with a total arsenic content of 3.36 mg/kg contained 0.10 mg/kg arsenite (As^{III}), 0.11 mg/kg arsenate (As^{V}), 0.35 mg/kg dimethylarsonic acid, 2.73 mg/kg arsenobetaine and 0.14 mg/kg arsenocholine. Atlantic cod (*Gadus morhua*) with a total arsenic content of 34.9 mg/kg contained 0.05 mg/kg arsenite (As^{III}), 0.09 mg/kg arsenate (As^{V}), 0.13 mg/kg dimethylarsonic acid, 33.2 mg/kg arsenobetaine and <0.03 mg/kg arsenocholine.

The level of arsenic in soil is dependent on the geological bedrock and the distance from sources of contamination. The concentration of arsenic in uncontaminated soils ranges from 2–10 mg/kg dry matter. Land used for agricultural purposes should not contain more than 20 mg/kg dry matter of arsenic. Arsenic in soil, unlike lead, cadmium and mercury, is quite mobile under neutral or slightly alkaline and reducing conditions. This relates to better solubility of trivalent arsenic compounds in comparison with pentavalent compounds. The mobility of arsenic in the soil determines its availability to plants. The transition coefficient of arsenic for soil–plant transfer is about 0.02–0.1. In areas with high rates of deposition and in areas where arsenic containing pesticides are applied, the predominant intake of arsenic by plants is foliar intake. Some crops (tobacco, cotton and oats) have a high tolerance to arsenic and concentrate it more than other plants.

6.5.3.2 *Occurrence in foods and dietary intake*

The total content of arsenic in selected foods is shown in Table 6.14. The toxic forms of arsenic include inorganic ions of

As(III) and As(V), methylarsonic and dimethylarsinic acids. High concentrations of arsenic are characteristic of seafood, especially of crustaceans and molluscs (oysters 3.7 mg/kg, squids 7.2 mg/kg, prawns 3.2–26 mg/kg, lobsters 1.5–122 mg/kg). In these foods, however, most of the arsenic occurs in almost non-toxic organic compounds, such as arsenobetaine (**6-21**) and arsenocholine (**6-22**). Higher amount of arsenic in foods of vegetable origin (tenth mg/kg) occur in oats and rice, and some wines can also contain higher amounts of arsenic. The normal levels of arsenic in wines range from 0.002 to 0.1 mg/l.

The tolerable daily intake of arsenic for an adult is 150 μg (at 70 kg body weight). According to earlier sources, the actual daily dietary dose of arsenic ranges from 17 μg (Canada 1987) to 130 μg (United Kingdom 1985). The major food contributors to arsenic exposure are fish and shellfish. For example, according to Belgian research (2009), the average total As concentrations in fish and shellfish species from the North Sea were 12.8 and 21.6 mg/kg, respectively. An average As concentration of 0.132 mg/kg was found in fish and 0.198 mg/kg in shellfish (approximately 10% of total As exposure from foods are the toxic As forms). Vegetables, fruit juices and fruits, rice, beer and wine and flour (wheat and maize) may also contribute to As dietary intake. Higher amounts may be found in wine if arsenic pesticides are used in the vineyard. In the last decade, the average daily dietary dose of arsenic in Western Europe countries has typically been in the tens of μg. In countries with increased consumption of fish and shellfish, a person eating seafood easily exceeds this value, as the average daily dietary dose can be about 300 μg.

6.5.3.3 Metabolism and toxic effects

The effectiveness of resorption of inorganic arsenic compounds in the gastrointestinal tract is about 5–25%. Organic arsenic compounds are apparently completely resorbed. Inhalation is also a route of exposure and a significant source is tobacco smoking, particularly when the tobacco was treated with arsenic-based pesticides. Owing to the high affinity for keratin, arsenic accumulates in hair and nails. The normal level of arsenic in hair is about 0.5 mg/kg. Inorganic arsenic compounds are metabolised in the body to methyl dimethylarsinic and methylarsonic acids (**6-40**) and are then excreted in the urine. The half-life of arsenic in the human body is 10–30 days. The toxicity of arsenic compounds decreases in the order: AsH_3 (arsine) > AsO_3^{3-} (*ortho*-arsenite), respectively, As_2O_3 (arsenic trioxide) > AsO_4^{3-} (arsenate) > methylarsonic acid > dimethylarsinic acid > arsenobetaine ≈ arsenocholine. The toxic effects of arsenic are related to its influence on the activity of important enzymes by binding to their thiol groups. Arsenic inhibits glutathione peroxidase, alanine aminotransferase, aspartate aminotransferase, glucose 6-phosphate dehydrogenase, cholinesterase, various phosphotransferases and enzymes with lipoic acid as the cofactor. In the glycolysis process, arsenate can replace inorganic phosphate, yielding 1-arseno-3-phosphoglyceric acid from glyceraldehyde 3-phosphate. Glyceraldehyde 3-phosphate is decomposed into 3-phosphoglyceric acid but 1,3-bisphosphoglyceric acid is not formed. Glycolysis proceeds, but one molecule of ATP produced from 1,3-bisphosphoglyceric acid is lost. Arsenate and other arsenic compounds also inhibit the citric acid cycle by blocking the conversion of pyruvate into acetyl-CoA, which results in further loss of ATP.

Chronic arsenic poisoning can occur even at a constant intake of 10 mg of arsenic per day. Large-scale accidental arsenic poisonings have occurred in the past. In the early 1900s, more than 6000 British beer drinkers were apparently poisoned with arsenic in the well-known Staffordshire beer epidemic in the United Kingdom. In 1955, more than 12 000 Japanese infants were poisoned, causing 130 deaths, when the sodium phosphate used as a stabiliser in infant formula preparations was contaminated with arsenic. More recently (in 1973), 11 cases of arsenic poisoning in western Minnesota, USA, were attributed to the consumption of water contaminated by an arsenic-based insecticide. Chronic poisoning involves loss of body weight, increased ptyalism and deterioration of vision. Typical skin changes include swelling, eczema and keratosis. Haematological and neurological changes can also occur (motor paralysis of the fingers, sleepiness, memory loss, confusion and impaired hearing). Arsenic also has carcinogenic, mutagenic and teratogenic effects.

The symptoms of acute poisoning with arsenic trioxide (As_2O_3) are abdominal pain, vomiting and diarrhoea. The skin is moist, and the pulse and breathing are weak and intermittent. A fatal dose for an adult is 70–180 mg, depending on their weight. Death occurs in 1–4 days. In the past, there have been some rare cases of acute poisoning in children by fruit treated with pesticides containing arsenic.

6.6 Toxic inorganic anions

In food, drinking water and drinks there are numerous inorganic anions. Some anions are forms of organogenic elements (such as carbonates and hydrogen carbonates), of essential elements (chlorides, phosphates, sulfates, iodides, fluorides, borates) and of non-essential elements (bromides). These and many other anions are mostly beneficial or harmless, and toxic effects exhibit only if present in food, drinking water and drinks in large quantities. Toxic effects can also result from an excessive accidental intake of these anions (fluorides, iodides and bromides).

Bromides have sedative and anticonvulsant properties. At high levels they replace chlorides in nerve transport mechanisms, stabilising the membrane and impairing nerve transmission. Bromide salt is an effective antiepileptic and sedative in small doses and was once a common ingredient of many medicines. Intoxication by bromides (bromism) is reported at serum levels as low as 400 mg/l, with 1500 mg/l considered to be toxic. The most common manifestations of bromism are psychiatric and dermatologic (bromoderma). Patients present with weakness, slurred speech, emotional instability, agitation, hallucinations, seizures and coma. Bromoderma occurs in approximately 35% of cases and is characterised by acneiform eruptions or, less commonly, granulomatous plaques, ulcers or bullae, usually on the face and trunk.

In addition to toxic mineral elements, some anions exhibit toxic effects on the human organism even if ingested in low concentrations. Legislation only specifies the maximum levels for nitrates and nitrites. Other anions, however, may also have toxic effects, such as

arsenates and arsenites (see Section 6.5.3.3), chromates and dichromates (see Section 6.3.13.1.2), perchlorates (see Section 6.3.2.2) and cyanides (see Section 10.3.2.3.1). Antinutritional effects are found in thiocyanates (rhodanides), which are metabolic products of cyanides or form by degradation of some glucosinolates occurring in certain vegetables in the mustard family (Brassicaceae), known as brassica or cruciferous vegetables (see Section 10.3.2.4.2).

Organic anions (such as citrate, malate and lactate) are absorbed mainly in the digestive tract and metabolised in various tissues, especially in the intestine and liver, and finally yield CO_2 and energy. The renal organic anion transport system is also an elimination route for many toxic organic anions (xenobiotics) as well as for endogenous organic anions (e.g. non-steroidal anti-inflammatory drugs, β-lactam antibiotics, cyclic nucleotidesand nucleoside analogues).

6.6.1 Nitrates and nitrites

Nitrates and nitrites are natural components of the environment and participate in the nitrogen cycle, a process of converting nitrogen into its various chemical forms. Important processes in the nitrogen cycle include fixation, mineralisation, nitrification and denitrification. The conversion of gaseous nitrogen from the atmosphere into a form available to plants (and hence to animals and humans) is associated with leguminous plants through their symbiotic association with *Rhizobium* bacteria and other bacteria (e.g. of the genus *Azotobacter*) that produce ammonia (NH_3) or ammonium ions (NH_4^+) using the enzymes known as nitrogenases. The soil nitrogen also comes from decaying plant and animal residues (crop residues, green manures and animal manure) containing proteins, nucleic acids and other nitrogenous substances, which are decomposed to ammonia by various microorganisms, such as bacteria and fungi. Ammonium salts and nitrates are used as commercial fertilisers. Nitrification is the process by which ammonium ions or ammonia are oxidised into nitrites (NO_2^-) by ammonia-oxidising bacteria (e.g. of the genus *Nitrosomonas*) and the nitrites are further oxidised into nitrates (NO_3^-) by nitrite-oxidising bacteria (of the genus *Nitrobacter*). The two processes of nitrification are called nitritation and nitratation. Denitrifying bacteria release nitrogen from nitrates, and nitrogen returns to the atmosphere.

The soil nitrogen occurs mainly in the form of ammonium ions and nitrates available to plants. Ammonium ions are retained in the soil since the cation is attracted to and held by the negatively charged soil clay and not leached to any great extent. The majority of the nitrogen used by plants is absorbed in the nitrate form. Heavy fertilisation leads to increased content of nitrates in the soil. However, nitrate is highly leachable and readily moves with water through the soil profile. If there is excessive rainfall or over-irrigation, nitrate will be leached below the plant's root zone and can eventually contaminate groundwater.

6.6.1.1 Occurrence

Nitrites and nitrates are an integral part of the nitrogen cycle in nature. Plants use nitrates from the soil to satisfy nutrient requirements and may accumulate them in their leaves and stems, and from there they can get into animal feed and human food. Nitrates and nitrites are likewise used as food additives. Nitrites act as nitrosation agents in reactions leading to the formation of toxic nitrosamines and other nitroso compounds in foods (see Section 12.2.7). Very high concentrations of nitrates and nitrites have been implicated in the appearance of methaemoglobinaemia and possibly in gastric cancer.

6.6.1.1.1 Foods of plant origin

The nitrate content in plants is strongly influenced by genetic and environmental factors. A large variability of nitrate content is observed between plant species and even between cultivars of the same species. Nitrate distribution is unequal between plant parts. Leaf blades have a lower content than stems and petioles, and young leaves show a lower nitrate concentration than older leaves. Accumulation of nitrates occurs in situations where the plant does not reduce nitrates to more easily assimilable ammonium salts. These situations specially include adverse humidity, temperature and insufficient light intensities that cause a lack of carbon compounds necessary for conversion of accumulated nitrates into amino acids and proteins. High irradiance reduces nitrate accumulation in plants. A decrease of nitrate accumulation in edible parts of plants during the day period and an increase during the night and under controlled irradiance in glasshouses, and also a correlation between plant water and nitrate concentration have been shown. Reducing the nitrate concentration in the nutrient solution or partly replacing nitrate with reduced compounds (such as ammonium salts or urea) lowers the plant nitrate content.

Vegetables (especially leafy vegetables, such as fresh lettuce and fresh spinach) and potatoes occupy a very important place in the human diet, but unfortunately constitute a group of foods that contributes maximally to nitrate consumption. Under excessive application of nitrogen fertiliser, these vegetables can accumulate high levels of nitrate and, upon being consumed, pose serious health hazards. Individual vegetables accumulate nitrates in varying quantities. According to their ability to accumulate nitrates, vegetables and root crops can be divided into three groups:

- vegetables with high concentrations of nitrates (>1000 mg/kg) such as lettuce, spinach, endive, chard (also known as mangold), Pekinensis and Chinese cabbages, radishes, small radishes, celery, rhubarb and sweet maize;
- vegetables with a medium content of nitrates (250–1000 mg/kg), which include cabbage, kale, cauliflower, eggplant, parsley, carrots, broccoli, garlic and potatoes;
- vegetables with low nitrate content (<250 mg/kg), which include Brussels sprouts, onions, tomatoes, peas, artichokes, asparagus and cucumbers.

The common nitrate content of some vegetables is listed in Table 6.15. In some crops, however, the nitrate content varies over a wide range (up to hundreds of per cent), due to genetic, climatic and soil conditions during the vegetation (such as light intensity,

Table 6.15 Levels of nitrates in important vegetables and potatoes.

Vegetable	NO_3^- content (mg/kg)		Vegetable	NO_3^- content (mg/kg)	
	Minimum	Maximum		Minimum	Maximum
Celery	0	3640	Bell pepper	4	330
Onion	0	1435	Parsley	0	5400
Garlic	44	2400	Leek	30	2159
Beans	14	717	Tomatoes	0	136
Pea	10	58	Rhubarb	300	2525
Savoy cabbage	0	3192	Radish (daikon, mooli)	300	3770
Brussels sprouts	0	2500	Radish	390	5200
Kohlrabi	80	4380	Red beet	45	4700
Cauliflower	0	2685	Lettuce	60	6600
Aubergine (eggplant)	71	960	Spinach	20	4500
Carrots	0	3337	Cabbage	0	3230
Cucumbers	0	490	Potatoes	0	2795

rainfall intensity and fertilisation). In fruits, nitrates are present in much smaller concentrations in comparison with vegetables. Only bananas and melons can have somewhat higher levels, of around 600–800 mg/kg.

6.6.1.1.2 Foods of animal origin

The natural content of nitrates in animal tissues is very low compared with plant tissues. The only exceptions are some meat products (such as ham and some sausages) and cheeses where nitrates or nitrites were used as additives in their manufacture.

Nitrates and nitrites are also used as food additives to produce a pink colour and a specific flavour in meat. This is the effect we see in cured meats. The nitrite used in meat curing is produced commercially as sodium nitrite. Sodium nitrite possesses antimicrobial properties that make it a good preservative. One special property of sodium nitrite is that it prevents the growth of the bacteria *Clostridium botulinum*, which produce several toxins that cause a paralytic illness that can lead to respiratory failure, which is seen in food born botulism (see Section 12.3.2.1). In some particular meat products, such as certain types of ham, sodium nitrate is used instead of sodium nitrite, because of the long aging period. Sodium nitrate is also used in the manufacture of some aged, semi-hard and hard cheeses (such as Emmental, Gouda and Edam) to prevent the late-blowing defect (characterised by eyes, slits and cracks caused by the production of gas bubbles), along with an aberrant and unwanted aroma, due to the growth of *Clostridium tyrobutyricum* bacteria that produce hydrogen, butyric acid and acetic acid as the major metabolites. The added sodium nitrate is reduced to nitrite, which inhibits the germination of bacterial spores.

6.6.1.1.3 Water

Nitrates and nitrites are present in all tap and bottled waters. They are produced by erosion of natural deposits and during the natural decay of vegetable material in soil. Rainfall washes these salts from the sub-soil into groundwater. Nitrogenous fertilisers used on arable farmland and leaks from septic tanks can be significant sources of nitrates in groundwater and surface water.

Many developed countries specify drinking water quality standards to be applied in their own country (such as the Drinking Water Directive in EU and Safe Drinking Water in the United States). For countries without a legislative or administrative framework for such standards, the World Health Organisation (WHO) publishes guidelines on the standards that should be achieved (see Section 7.2.3.1). The following standards are included in the Drinking Water directive: 50 mg/l nitrate (as NO_3^-) and 0.5 mg/l nitrite (as NO_2^-) to ensure that drinking water will not cause methaemoglobinaemia. Maximum Contaminant Levels (MCLs) set for nitrates and nitrites by the US Environmental Protection Agency (EPA) for drinking water quality are: nitrates 10 mg/l (as nitrogen), nitrite 1 mg/l (as nitrogen).

6.6.1.2 Health and toxicological evaluation

The content of nitrates in food is regulated in the EU and also in many other countries. Limits for their content in foods in the EU are shown in Table 6.16. At typical concentrations, nitrates are relatively non-toxic for adults because they are relatively rapidly excreted in the urine. The ADI (Acceptable Daily Intake) value, which quantifies the daily amount of nitrates in food that a person can ingest over a lifetime without a health risk, is 3.7 mg/kg body

Table 6.16 Maximum levels for nitrates in foods.[a]

Product	Maximum levels in mg/kg	
Fresh spinach[b]	Harvested 1 October to 31 March	3000
	Harvested 1 April to 30 September	2500
Preserved, deep-frozen or frozen spinach		2000
Fresh lettuce (protected and open-grown) excluding iceberg-type lettuce	Harvested 1 October to 31 March	
	lettuce grown under cover	4500
	lettuce grown in the open air	4000
	Harvested 1 April to 30 September	
	lettuce grown under cover	3500
	lettuce grown in the open air	2500
Iceberg-type lettuce	Lettuce grown under cover	2500
	Lettuce grown in the open air	2000
Processed cereal-based foods and baby foods for infants and young children[c]		200

[a] Official Journal of the European Union, 20 December 2006, L 364/5. Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.

[b] The maximum levels do not apply for fresh spinach to be subjected to processing and which is directly transported in bulk from the field to processing plant.

[c] The maximum level refers to the products ready to use (marketed as such or after reconstitution as instructed by the manufacturer).

weight. However, their potential toxicity results from the possibility of their reduction to nitrites (ADI = 0.07 mg/kg body weight).

Nitrates are partially reduced by the nitrate reductase of microorganisms present in raw materials during transport, storage and processing of vegetable raw materials. Nitrites also form endogenously in the digestive tract by the action of microorganisms. The ingested nitrates are excreted in the urine (about 80%, or about 50% in older people) in 4–12 h, and the rest remains in the body. It is expected that the nitrates remaining in the digestive tract are mainly transformed into ammonium salts. The toxic effect of nitrites, after their absorption into the blood, depends upon their possibility of inducing methaemoglobinaemia. Acquired methaemoglobinaemia is a blood disorder in which an abnormal amount of methaemoglobin (containing Fe^{3+}) is produced by the oxidation of haemoglobin (containing Fe^{2+}) and methaemoglobin is unable to transport oxygen effectively to body tissues (see Section 9.2.1.5.1). Under normal physiological conditions, the amount of methaemoglobin is about 2%. Methaemoglobin reductase in red blood cells of an adult converts the methaemoglobin created back into haemoglobin, but this enzyme may be produced in insufficient amounts or be absent in certain people that have an inherited mutation.

Nitrites are especially dangerous in infants during the first 2–4 months (or even up to 6 months) of life and may cause methylhaemoglobinemia, known as blue baby syndrome. During this period, their corresponding enzyme system is not sufficiently developed. The amount of foetal haemoglobin (haemoglobin F) in infants is about 85% of the total haemoglobin amount and foetal haemoglobin is more easily oxidised than haemoglobin of adults (haemoglobin A). In infants, the stomach also has a lower concentration of acids (higher pH), so there non-pathogenic microorganisms may occur and reduce nitrates to nitrites before nitrates resorb. Nitrates can also partly move to the salivary glands, where they concentrate and re-enter the oral cavity. Endogenous reduction to nitrites already takes place in the mouth, both in children and even adults. This will make up to 65% of the total content of nitrites. The external manifestation of methaemoglobinaemia is the gray–blue to blue–violet colour of mucous membranes, skin and peripheral parts of the body, especially around the eyes and mouth. The first symptoms appear when the methaemoglobin concentration in the blood reaches 6–7%. After the age of 6 months, methaemoglobinaemia is not a threat since the nitrate converting bacteria are no longer present in the baby's stomach. For these reasons, the level of nitrates and nitrites in water and foods for infants and young children is regulated.

6.7 Radionuclides

6.7.1 Radionuclides and radioactivity

Radionuclides are **nuclides** with an unstable nucleus, which are subject to radioactive decay. According to the type of radionuclide, this radioactive nuclei decay creates three main types of radiation (radioactive decay):

- α-decay, a stream of particles composed of two protons and two electrons (identical with the nucleus of helium ^{4}He);

- β-decay, a stream of electrons ($_{-}\beta$) or positrons ($_{+}\beta$) of high speed (about 1×10^8 m/s);
- γ-radiation (γ-rays), an electromagnetic radiation of high frequency ($>1\times10^{19}$ Hz) and short wavelength (<1 nm) that accompanies emissions of α- and β-particles.

Radionuclides can be roughly divided into α-emitters and β-emitters. The emission of α-particles is associated with a decrease in mass number by four units and in the atomic number by two units. The loss of two protons results in an excess of two electrons, so the α-decay is accompanied by the emission of electrons (radiation $_{-}\beta$). In the radioactive β-type conversion, the nucleon in the atom nucleus transforms, a neutron turns into a proton or vice versa, which is associated with the change of particle charge and emitting an electron or positron. The capture of an electron (usually from the sphere K of the electron cloud) by the atom nucleus is also possible, while changing a proton into a neutron. In the electron cloud, the electron from the higher level then jumps to fill the vacant place, which is accompanied by the emission of a photon.[4]

6.7.2 Sources of radioactivity

All the heavy elements, starting from the element with atomic number 84 (polonium, Po), have at least one radioactive nuclide (isotope). However, a number of light elements also form radioactive isotopes (such as tritium ^{3}H, carbon ^{14}C and potassium ^{40}K). In nature there are three types of radionuclides:

- heavy nuclides
- their daughter products (daughter nuclides)
- light nuclides.

The first group includes very heavy elements such as uranium (U) or thorium (Th). Radionuclides of these elements have an extremely long half-life (e.g. ^{232}Th 1.41×10^{10} years, ^{238}U 4.51×10^{9} years, ^{235}U 7.1×10^{8} years). The second group includes daughter nuclides formed by the radioactive decay of the first group of nuclides. For example, radium ^{226}Ra (half-life of 1600 years) arises by decay of uranium ^{238}U. The decay of radium yields the radionuclides radon ^{222}Rn, polonium ^{210}Po and lead ^{210}Pb. Natural heavy nuclides represent three decay chains. A parent isotope is one that undergoes α- or β-decay to form a daughter isotope. The daughter nuclide may be stable or it may decay to form a daughter nuclide of its own. Each decay chain thus has its initial mother nuclide and a final stable nuclide. By tracking the interrelationships of these nuclides, the radioactive nuclides are arranged into the following series:

- thorium series: begins with thorium ^{232}Th and ends with lead ^{208}Pb;
- uranium series: begins with uranium ^{238}U and ends with lead ^{206}Pb;
- actinium series: with so called actinouranium ^{235}U and ends with lead ^{207}Pb.

Another group of radionuclides is generated by cosmic rays in the light elements in the atmosphere. For example, nitrogen forms radioactive carbon ^{14}C. The natural radioactivity in nature is mainly due to radionuclides of light elements. Approximately 90% of the radioactivity is attributable to ^{40}K (it represents 0.012% of natural potassium and has a half-life of 1.3×10^{9} years) and almost all the rest to carbon ^{14}C (half life of 5730 years). Increased radioactivity in certain areas can be caused by abnormally high incidence of radioactive elements (especially uranium) in rocks.

The anthropogenic sources of radioactivity in the environment include the testing of nuclear weapons and radioactive material handling, especially in nuclear power plants. In the explosion of atomic bombs or in nuclear reactors, a complex mixture of different radionuclides is produced, namely uranium ^{235}U, plutonium ^{239}Pu, caesium ^{137}Cs (half-life of 30 years), strontium ^{90}Sr (half-life of 28 years), cobalt ^{60}Co (half-life of 5.3 years), caesium ^{134}Cs (half-life of 2 years), ruthenium ^{106}Ru (half-life of 1 year) and iodine ^{131}I (half-life of 8 days). A number of other radionuclides result from an atomic explosion by collision of neutrons with the atoms of elements that are contained in the casing of the non-explosive parts of the atomic bomb. For example, these activation products include zinc ^{65}Zn (half-life of 245 days).

6.7.3 Content of radionuclides and radiation dose

The content of radioactive nuclides in environmental materials and in foods is usually expressed as the specific activity given most often in Bq/kg and for liquid materials in Bq/l. The dose of radiation absorbed is expressed as the mean energy absorbed per unit of weight or per unit of volume of material. The basic unit of the

[4]Radioactive decay of nuclei is a first-order reaction; decay rate (activity A) is therefore dependent on the concentration (content) of the radionuclide and is the product of this concentration (more precisely, the number of atoms of radionuclide N) and the decay constant λ (in s^{-1}): $A = -(dN/dt) = \lambda.N$. The basic unit of activity, according to the System International (SI system) is the Bq (becquerel). One Bq (in s^{-1}) is defined as the activity of a quantity of radioactive material in which one nucleus decays per second. Previously, the frequently used unit was the Ci (curie) defined as 3.7×10^{10} decays per second. For conversion, the following relationship can be used: 1 Ci $= 3.7\times10^{10}$ Bq. Number of radionuclide atoms transformed in time t is: $N = N_0.e^{-\lambda t}$, where N_0 is the initial number of atoms of the radionuclide at the time $t = 0$. During conversion, the number of radioactive atoms of the radioactive nuclide is continuously decreasing. Combining both equations we get the relation expressing the dependence of activity on time: $A = -(dN/dt) = \lambda.N_0.e^{-\lambda t}$. The period of time it takes for the amount of a radionuclide undergoing decay to decrease by half is called the half-life, abbreviated to T (or $t_{1/2}$), which is inversely proportional to the decay constant: $T = \ln2/\lambda = 0.69315/\lambda$. After the time equal to ten times the half-life, 99.9% of the radionuclide is transformed.

radioactive dose[5] is the gray (Gy); one Gy (in J/kg) is defined as the absorption of one joule of ionising radiation by one kilogram of material.

The individual types of ionising radiation resulting from nuclear processes have different biological effects. Therefore, in addition to the exposure dose (given in Gy), the so-called equivalent dose, absorbed by a given mass of biological tissue, was introduced. The equivalent dose (H) for tissue T and radiation type R is calculated by the formula: $H_{T,R} = Q.D_{T,R}$, where Q is a radiation (radiobiological) quality factor that depends on the type and energy of that radiation, $D_{T,R}$ is the total energy of radiation absorbed in a unit mass of any material. The quality factor Q is also known as the relative biological effectiveness of the radiation. For example, β- and γ-radiation has a value of $Q = 1$, α-radiation has a value of $Q = 10$, radiation quality factor of the neutron flux varies, depending on the neutron energy, from 2.5 to 10. While the unit gray (Gy) measures the absorbed dose of radiation (D) by any material, the sievert (Sv, in J/kg), unit of dose equivalent radiation, measures the equivalent dose of radiation (H) and evaluates the biological effects of the ionising radiation. It means that the dose of α-radiation of 1 Gy corresponds to the dose equivalent radiation of 10 Sv. The same dose equivalent radiation has a dose of γ-radiation of 10 Gy. The load of the organism due to natural radioactivity is around 2 Sv per year, of which about 0.4 mSv is due to radionuclides occurring in food.

To assess the radiation hazard to humans, it is necessary to take into account several factors:

- radionuclide species, including the possibility of its conversion into other radionuclide and the related type of radiation;
- specific activity of the environment (food);
- type and length of exposure (external irradiation, internal irradiation by ingestion of radioactive food or inhalation of radioactive aerosols);
- chemical properties of radionuclides (in the case of internal exposure) that affect their behaviour in the body; their chemical analogy with essential elements and similar reactivity results in the transportation to certain target organs, where they accumulate (e.g. accumulation of radioactive iodine ^{131}I in the thyroid gland, analogy of radioactive caesium ^{134}Cs with ^{137}Cs as well as of strontium ^{90}Sr with potassium and calcium).

According to the potential hazard, radionuclides are classified into four groups with:

- very high radiotoxicity (^{90}Sr, ^{210}Pb, ^{211}At, ^{226}Ra, ^{227}Ac, ^{239}Pu, ^{241}Am, ^{242}Cm; deposited mainly in the bones)
- high radiotoxicity (^{45}Ca, ^{59}Fe, ^{89}Sr, ^{131}I, ^{140}Ba, ^{234}Th, ^{238}U)
- medium radiotoxicity (^{22}Na, ^{24}Na, ^{32}P, ^{35}S, ^{36}Cl, ^{42}K, ^{60}Co, ^{132}I, ^{137}Cs)
- low radiotoxicity (^{3}H, ^{7}Be, ^{14}C, ^{18}F).

6.7.4 Occurrence in environment and in foods

A substantial part of the natural radioactivity is produced by nuclides of light elements ^{40}K and ^{14}C that are dispersed in the nature along with the major isotopes of potassium and carbon. Heavy radionuclides are not uniformly distributed in nature. For example, around 99.284% of natural uranium is ^{238}U that is strongly bound in some rocks (granite). Given the very long half-life (4.468×10^9 years), the effect of ^{238}U in the environment is permanent. The decay of this isotope creates a number of other radionuclides that are generated via ^{234}Th (half-life 24 days) and ^{234}Pa (half-life 1.2 min) and have long half-lives: ^{234}U (245 thousand years), ^{230}Th (75 000 years) and radium ^{226}Ra (1600 years). Radium gives, by α-decay, the radioactive noble gas radon ^{222}Rn with a half-life of 90 hours. The radon activity contributes significantly to the exposure of people in an indoor environment of some buildings, such as those that are thermally insulated from its surroundings using materials containing granite. Because it can be inhaled, a prolonged exposure to radon is quite dangerous. Other members of the decay series are mostly nuclides with short or very short half-lives. The only exceptions are lead ^{210}Pb (22 years) and polonium ^{210}Po (138 days). The stable end product of a decay of uranium ^{238}U is lead ^{206}Pb.

Mining and processing of minerals, ores and fossil fuels are activities that bring, in addition to other toxic substances, radioactive elements into the environment. Handling of nuclear materials, nuclear accidents and nuclear weapons tests also contribute to the total radioactivity in the environment. The most famous incident was the atomic power plant disaster in Chernobyl, Ukraine, which occurred on 26 April 1986. From 1986 to 2000, over 350 000 people were evacuated and resettled from the most severely contaminated areas. Leakage of the radioactive material (with a predominance of ^{131}I, ^{137}Cs and ^{132}Te) into the environment was to such an extent that there was significant contamination not only of the area of the atomic power plant, but due to atmospheric transfer, the radioactivity was spread throughout Europe and parts of Asia. The total radioactivity released as a result of this nuclear disaster is estimated at 3×10^{18} Bq. In order to minimise public exposure to radioactive elements (mainly ^{131}I), Poland and some Western European countries banned the distribution of milk originating from cows grazing freely, and children preventively received non-radioactive potassium iodide. This event significantly increased the interest in studying the behaviour of radionuclides in the environment. In the 1980s and 1990s, as a consequence of this nuclear disaster, radioactivity was monitored in different compartments of the environment virtually worldwide. Significant attention has been paid to long-range transport of radioactive aerosols, to radioactive

[5] Conversion of immediate activity, specific for the given nuclide, to the nuclide mass (m, in grams) is given by the equation: $m = A.M/\lambda.N_A$, where A = nuclide activity (in Bq), M = molar nuclide mass (in g/mol), N_A = Avogadro's number, $N_A = 6.022\ 141\ 527 \times 10^{23}$ per mole. Another unit for the radioactive dose is eV/g or eV/cm^3. The electronvolt (eV) is by definition equal to the amount of kinetic energy gained by a single unbound electron when it accelerates through an electric potential difference of 1 volt (V), $1\ \text{eV} = 1.6021 \times 10^{-19}$ J.

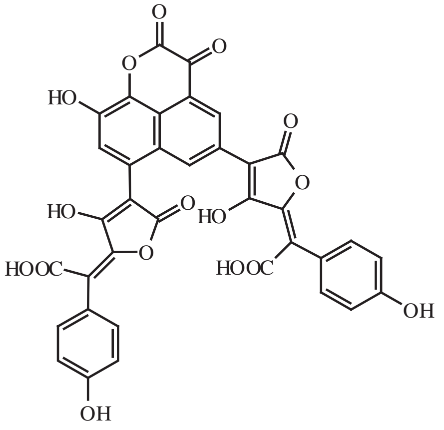
fallout, to contamination of soil and to the possibility of transport of radionuclides from soil to plants.

The mobilities of radionuclides in environmental compartments are determined by the chemical properties of the element, and these mobilities are higher, for example, for halogens (e.g. ^{129}I, ^{131}I and ^{36}Cl), alkali metals (^{40}K, ^{134}Cs, ^{137}Cs), non-metals (^{89}Se) and alkaline earth metals (^{90}Sr) than for transitional metals and especially for transuranium elements. Plants are partially contaminated by direct deposition on the leaves and partly by intake of radionuclides from the soil, which depends on the binding of the radionuclide in the soil components, depth of the root system and developmental stage of plants at the time of deposition. Experience with the transfer of caesium radionuclides from soil to plants and fungi after the Chernobyl disaster can be summarised as follows:

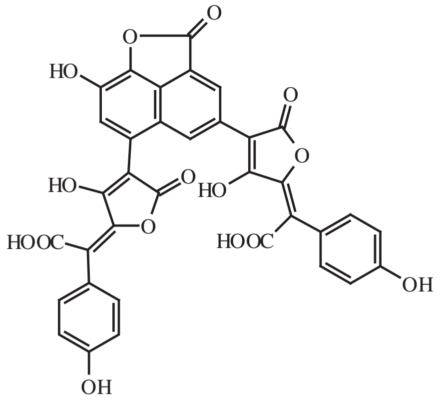
- alfalfa, which has deep roots, was not even in the second generation contaminated by fallout, unlike grasses and wild herbs, which are rooted largely in the surface soil layer;
- compared with the spring cereals, winter cereals had contamination that was roughly twice as high;
- summer fruits (such as currants) were contaminated more than late fruits (apples);
- needles of conifers (often used as bioindicators of pollution) showed the specific activity of caesium ranging from 1×10^2 to 1×10^3 Bq/kg, the fungi and lichens showed values up to one order of magnitude higher (1×10^2 to 1×10^4 Bq/kg).

The Chernobyl disaster in 1986 resulted in substantial radioactive fallout in parts of Norway. The deposition of radioactive dust caused contamination by caesium ranging from 5 to 200 kBq/m^2. In some areas of Czechoslovakia, the contamination with radioactive caesium was higher than 10 kBq/m^2; in other areas it reached only 2–3 kBq/m^2. In different areas of Finland, the transfer of ^{137}Cs and ^{90}Sr from soil to some crops was studied for several years. Radioactive caesium follows the path of potassium and tends to accumulate in plant tissues, including fruits and vegetables. The ratio of specific activities of radionuclides in plants and soil ranged from 0.01 to 2.29 for ^{137}Cs and from 0.02 to 2.44 for ^{90}Sr (values in subsequent years gradually decreased). This value for ^{137}Cs decreased in the individual crops as follows: lettuce, cabbage > carrots, potatoes and onions > cereals. The smallest transition of ^{137}Cs to plants was recorded in the crops grown on clay soils. The order for ^{90}Sr was: lettuce, cabbage > carrots, onions > cereals > potatoes.

Small amounts of ^{134}Cs and ^{137}Cs are released into the environment during nearly all nuclear weapon tests and some nuclear accidents, most notably the Chernobyl disaster. It is well documented that mushrooms, including edible species from contaminated forests, accumulate radionuclides (mainly ^{137}Cs) in their fungal sporocarps. For example, the edible mushroom commonly known as penny bun, porcino or cep (*Boletus edulis*) from Poland (1987) showed about seven times higher activity of ^{137}Cs in comparison with the radioactive background and about double the activity of mushrooms from the United States. This activity (1100 Bq/kg) corresponded to the concentration of ^{137}Cs of around 0.0003 ng/kg, which is negligible in comparison with the content of stable caesium (^{133}Cs). To achieve radiotoxic doses, it would be necessary to consume 3000 kg of dry mushrooms. In addition it was found that radioactive caesium can be effectively removed from the mushroom by washing with water or salt solution. High concentrations of radionuclides found in the fruiting bodies of the bay bolete *Boletus badius* (Boletaceae) after the nuclear reactor accident at Chernobyl have been attributed to the complexation of ^{137}Cs by the so-called naphthalenoid pulvinic acids that occur as their dipotassium salts in the cap skin of this toadstool. The main compounds are badione A (**6-41**) and norbadione A (**6-42**), which are responsible for the chocolate brown and golden yellow colours of the cap skin of this bolete and related bolete species. For example, complexation of ^{137}Cs with norbadione A involves a mixture of *Z*/*E* isomers and conformers of norbadione A with a broad diversity of binding modes. In the mycelia ^{137}Cs is mostly trapped by polyphosphates in vacuoles and other organelles.



6-41, badione A



6-42, norbadione A

The content of radioactive caesium in milk and meat of livestock depends mainly on the contamination of the feed. For example, cows resorb about 30% of radioactive caesium in the feed. In 1986,

Czechoslovakia restricted the pasture of cows and their nutrition was partly ensured using preserved feed from the previous year. Specific activity of ^{137}Cs in beef in 1986 ranged from 14 to 129 Bq/kg. The maximum value observed in 1986 was 240 Bq/kg in milk, and most samples did not exceed a level of 40 Bq/kg. In subsequent years, there was a significant decline (in 1988 only 0.7% of milk samples exceeded the level of 20 Bq/kg).

Significantly higher specific activity of caesium was detected in the meat of game (in 1986, depending on surface contamination of the territory, the observed values for roe deer meat were 120–600 Bq/kg and 170–660 Bq/kg for deer meat). Contamination of wild life by radionuclides decreases in the order: deers > roe deers and fallow deers > mouflons > wild boars (wild pigs) > hares.

As regards the dietary intake of radionuclides, the average exposure of an adult amounting to approximately 1.7 mGy/year was found in Germany in the 1970s. The amount of 0.2 mGy was accounted for by natural radionuclides (mainly ^{40}K and ^{14}C). The total intake of radionuclides ^{90}Sr and ^{137}Cs was in units of Bq per person per day. As a result of the Chernobyl disaster in 1986, the following year-long dietary intake of radionuclides were: 4600 Bq ^{131}I, 1760 Bq ^{134}Cs, 3400 Bq ^{137}Cs and the total dose equivalent increased by 0.04–0.26 mSv.

6.7.5 Fate in organism

After the radionuclides have entered into the body of an animal or human by ingestion or inhalation, distribution to individual tissues and organs and their partial incorporation into these tissues takes place. For example, radioactive caesium as an analogue of potassium, after entering into the body quickly appears in the kidney, liver and spleen and accumulates in muscle tissue. As with other alkali metals, the excess of radioactive caesium washes out of the body relatively quickly in sweat and urine. Radioactive iodine concentrates in the thyroid gland. Heavy radioactive metals accumulate in internal organs and bones. Subsequently, these metals are excreted and their concentration also decreases due to radioactive decay. The time required for the radioactivity of material taken in by a living organism to be reduced to half its initial value by a combination of biological elimination processes and radioactive decay is called the effective half-life $T_{ef} = T_f T_b/(T_f + T_b)$; T_f is the physical half-life of a radionuclide and T_b is the biological (elimination) half-life of the element in the given form.

6.7.6 Legislation and regulation

Radiocaesium is one of the most important artificial radionuclides produced by nuclear fission. Consumption of agricultural products contaminated with radiocaesium represents the principal route of human exposure to this radionuclide. Within the EU, the limits of activity of ^{134}Cs and ^{137}Cs (that is a strong emitter of γ-radiation) of 370 Bq/kg have been established for milk and foods for children. For other food imported into the European Union, a limit of 600 Bq/kg has been set.

7
Water

7.1 Introduction

Water is one of the most widespread substances in the biosphere. In food chemistry, water is one of a group of nutrients (together with proteins, lipids, carbohydrates, vitamins and minerals) that are necessary for the normal functioning of living organisms. Owing to its physico-chemical properties, water is particularly important:

- in temperature regulation of living organisms
- as a transport medium of nutrients, metabolic products and respiratory gases
- as a solvent or dispersion medium
- as a reactant involved in biochemical and chemical reactions.

People's lives and livelihoods depend on water. The normal functioning of the human body inevitably leads to a continuous loss of water that must be compensated by the water produced via the oxidation of primary nutrients: proteins, carbohydrates and lipids. This water is sometimes referred to as **endogenous** or **metabolic water**. The amount of endogenous water is not sufficient to cover the water lost, and the organism has to compensate with **exogenous water** contained in foods and especially in drinks. It is reported that the daily output of water by an adult is approximately 2500 g, of which about 1500 g (minimum of 600 g) are excreted in the urine, with typically about 550 g of water excreted through the skin (sweating), 350 g of water are exhaled into the air and 100 g in faeces. Replacement of the same amount of water is met by exogenous water in the form of various beverages (about 1300 g) and foods (about 900 g), and approximately 300 g of water are obtained by oxidation of the primary nutrients, proteins, lipids and carbohydrates (endogenous water). Oxidation of 1 g of proteins, for example, gives 0.37 g, oxidation of 1 g of fat gives 0.4 g and oxidation of 1 g of carbohydrates (glucose) yields 0.6 g of water. Foods are very important sources of water, a fact that is often overlooked – we tend to concentrate only on the importance of drinking water and on water present in beverages.

This chapter deals, in the first part, with the production of drinking water and its quality requirements. Other parts of the chapter present the water content of foods, and the major changes to the water content that occur during culinary and technological operations. A substantial portion of the chapter is devoted to interactions of water with food components (inorganic compounds, proteins, carbohydrates and lipids) and various phenomena that occur on the phase interfaces (surface tension, adsorption and capillary phenomena). Various food dispersal systems are described (sols, gels, emulsions and foams), where the presence of water significantly affects the texture of the foods. The last part of the chapter deals with water activity in foods and its influence on microorganisms, biochemical and chemical reactions and the organoleptic properties of foods.

7.2 Drinking water

Water is renewable; it exists in an endless cycle, moving between its gaseous, liquid and solid forms. This **hydrological cycle** constitutes nature's way of replenishing, redistributing and purifying the world's natural water resources. In absolute terms, the total renewable freshwater resource in Europe is around 3500 km^3 per year. However, the balance between water demand and availability has reached a critical level in many areas of Europe as the result of over-abstraction and prolonged periods of low rainfall or drought. The Mediterranean islands of Malta and Cyprus and the densely populated European countries (Germany, Poland, Spain, England and Wales) have the least available water per capita.

Drinking (potable) water comes from **surface water** and **ground water** resources. Surface water resources, including rivers, lakes and reservoirs, are the largest and most reliable of all freshwater resources in Europe. For example, the surface water abstraction was

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91% in the Netherlands (in 2008), 82% in Germany and France (in 2007) and Spain (in 2008), 81% in the Czech Republic (in 2009), 78% in Poland and 74% in England and Wales (in 2008). In many areas most drinking water is groundwater, up to 100% in Malta, 98% in Denmark and 79% in Cyprus (in 2009), about 80% in Russia and even more in North Africa and the Middle East.

Natural water is never chemically pure. According to the origin, various substances are dissolved and sometimes suspended in the water. The quality of surface waters, as opposed to ground water, depends on many factors. In comparison with ground waters, surface waters usually have much higher concentrations of organic substances of various origins, contain higher amounts of dissolved oxygen, lower levels of carbon dioxide and have low concentrations of iron and manganese ions. The level of microorganisms in surface waters is significantly higher than in ground waters.

7.2.1 Classification

Surface waters are categorised into five classes according to quality. Classification is based on the evaluation of mandatory water quality indicators that are guides to the oxygen regime (the amount of dissolved oxygen), basic chemical and physical parameters (pH, dissolved substances, conductivity, suspended solids, ammonia nitrogen, nitrate nitrogen and total phosphorus), additional parameters (calcium, magnesium, chlorides, sulfates, anionic surfactants, hydrocarbons and organically bound chlorine), heavy and toxic elements content (lead, cadmium, mercury and arsenic), biological and microbiological parameters (especially coliform bacteria) and indicators of radioactivity. The five classes of surface water are:

- Class 1 is an extra-clean fresh surface water resource, not necessarily required to pass through a water treatment process and needing only basic treatment for the destruction of pathogens.
- Class 2 is a very clean fresh surface water resource used for consumption, which requires basic water treatment processing before use.
- Class 3 is a medium-clean fresh surface water resource used for consumption, which requires passing through a basic treatment process before use in agriculture.
- Class 4 is a fairly clean fresh surface water resource used for consumption, but requires a special water treatment process before being used by industry.
- Class 5 is surface water, which is not classified in classes 1–4.

Extra-clean water (class 1) is suitable for all uses, mainly for consumption (drinking water) and use in the food industry. Very clean water (class 2) is usually adequate for most applications (e.g. for water supply purposes). Classes 3–5 waters are usually only suitable for supplying industrial plants.

Ground waters are divided according to the quality of water suitable for consumption (good status) and water inappropriate for consumption (poor status). Ground waters suitable for consumption can be clearly distinguished from natural mineral waters by their nature, which are characterised by the mineral content, trace elements or other constituents and, sometimes, by certain physiological effects. Local standards differ by country; nevertheless ground waters are often divided into two categories:

- spring waters (with a minimum of 1000 mg of total solids or a minimum of 250 mg of free carbon dioxide);
- mineral waters (where the dissolved solids content is higher than 1000 mg/l).

In accordance with European legislation, natural mineral water is defined as microbiologically wholesome water from an underground aquifer. Natural mineral waters are evaluated, for example, in the Czech Republic according to:

(i) the total mineralisation as mineral waters:
  - very slightly mineralised (dissolved solids content is lower than 50 mg/l)
  - slightly mineralised (dissolved solids content is 50–500 mg/l)
  - moderately mineralised (dissolved solids content is 500–1500 mg/l)
  - highly mineralised (dissolved solids content is 500–5000 mg/l)
  - strongly mineralised (dissolved solids content is higher than 5000 mg/l)

(ii) the content of dissolved gases and of important components as mineral waters:
  - carbonic (CO_2 content is higher than 1 g/l)
  - sulfuric with contents of sulfur (sulfan (i.e. hydrogen sulfide) dissociated to various degrees and thiosulfates) higher than 2 mg/l
  - iodine (iodide content is higher than 5 mg/l)
  - others, such as waters with increased content of silicic acid (above 70 mg/l) and fluorides (above 2 mg/l)

(iii) the actual pH, only in the case that water is:
  - strongly acidic (with pH below 3.5)
  - strongly alkaline (with pH above 8.5)

(iv) radioactivity of radon, waters with radioactivity above 1.5 kBq/l caused by the radioactive gas radon (^{222}Rn)

(v) spring temperature into:

- cold waters (with temperature up to 20 °C)
- tepid waters (with temperature up to 35 °C)
- warm waters (with temperature up to 42 °C)
- hot waters (with temperature above 42 °C)

(vi) osmotic pressure into:

- hypotonic (with osmotic pressure lower than 710 kPa)
- isotonic (with osmotic pressure 710–760 kPa)
- hypertonic (with osmotic pressure above 760 kPa) and also by other properties.

7.2.2 Production

Most water requires some type of treatment before use. Depending on the type (surface water or ground water) and quality of the water resource, the production of drinking water is achieved by various technological processes. Some water resources directly meet the requirements for drinking water, while other resources only require disinfection, aeration or deacidification (removal of dissolved carbon dioxide and oxygen). Treatment processes typically consist either of chlorine disinfection only, or of direct or conventional filtration and chlorination. Surface water sources from protected catchments are typically treated by chlorination only and those from impacted catchments by conventional coagulation, flocculation, sedimentation, filtration and chlorination. Some resources require more complex treatment of the water, such as increasing the concentrations of Ca^{2+} and HCO_3^- ions (in soft water), reducing the concentration of Fe^{2+} and Mn^{2+} ions (using special manganese removal equipment), removal of NH_4^+ ions, heavy metals, radioactive substances (radon and radionuclides) and other treatments. Groundwater sources are typically treated by aeration and chlorination.[1] These issues are addressed in detail in water technology books.

7.2.3 Quality requirements

7.2.3.1 General requirements

Drinking water contains varying amounts of inorganic salts (cations and anions formed by dissociation of various salts), dissolved gases (carbon dioxide, oxygen and other gases) and indicators of pollution, for example soluble portions of humus from the soil (called humins or humic substances) and contaminants (e.g. phenols and petroleum fractions) and various bacteria. The presence of carbon dioxide and oxygen can increase the number of ions present; if the water is in contact with metals (copper, iron and other metals) it can corrode kitchen and manufacturing equipment. Water containing high concentrations of iron and manganese ions is useless for many purposes. The presence of bicarbonates, sulfates and chlorides of calcium and magnesium has practical significance in determining **water hardness** and also its suitability and applicability for the production of foods and also for other purposes. Water hardness is assessed according to the level of bicarbonates (temporary hardness, also known as carbonate hardness) and those of sulfates and chlorides (permanent hardness or non-carbonate hardness). Water hardness is often expressed in German degrees (°dH or dGH), American degrees (mg/l), English degrees (°e or °Clark) or French degrees (°f). For example, 1°dH corresponds to 10 mg of CaO or 7.17 mg of MgO in 1 litre, 1°e corresponds to 64.8 mg of $CaCO_3$ per 4.55 litres, 1°f corresponds to 10 mg $CaCO_3$ per litre ($1°dH = 1.252°e = 0.5603°f$). Water with a hardness of 0–7°dH is known as soft water, with 7–14°dH as moderately hard water, with 14–21.3°dH as hard water and with >21.3°dH as very hard water. Temporary hardness can be removed by boiling or by adding calcium hydroxide. Calcium (or magnesium) bicarbonate is thus transformed into insoluble carbonate during cooking:

$$Ca(HCO_3)_2 \rightarrow CaCO_3 + H_2O + CO_2$$

Calcium hydroxide reacts as follows:

$$Ca(HCO_3)_2 + Ca(OH)_2 \rightarrow 2\ CaCO_3 + 2H_2O$$

Removal of temporary and permanent hardness in practice can be done using ion exchangers or by the addition of sodium carbonate:

$$CaSO_4 + Na_2CO_3 \rightarrow CaCO_3 + Na_2SO_4$$

Quality requirements for drinking water include microbiological, biological, physical, chemical and radiological aspects. Some of these quality requirements are shown in Table 7.1.

Bottled water is a selected high-quality ground water suitable for continuous use by the population. The only treatments allowed prior to the bottling of waters are some physical processes (chemical processes are used only rarely) to remove unstable components, such as iron and sulfides and to (re)introduce carbon dioxide. To protect the biological and microbiological quality, chlorine and its compounds should not be used. Bottled waters, other than those labelled 'natural mineral water', are expected to conform to essentially the same standards as the public water supply and they are therefore suitable for giving to infants or for preparing feeds. As with tap water, bottled waters should be boiled and cooled before using to make up infant formula feeds. Local standards differ by country. In the Czech Republic, for example, bottled water for infants is not modified by any of the procedures described above. The hygienic quality asurance is possible only by ultraviolet radiation

[1] The European Parliament and the Council have established a framework for Community action in the field of water policy, known as the Water Framework Directive. The Drinking Water Directive relates specifically to water intended for human consumption For example, in the United Kingdom the Water Quality Regulations prescribe maximum values for substances that affect wholesomeness and the Drinking Water Inspectorate polices the water companies. In the United States, the Environmental Protection Agency (EPA) sets standards for tap and public water systems under the Safe Drinking Water Act (SDWA).

Table 7.1 Selected quality requirements for drinking water.

Parameter (units)	Value	Parameter (units)	Value
Indicator		Chromium (μg/l)	50
Colour, odour, taste, turbidity	–[a]	Copper (mg/l)	2
Conductivity (μS/cm at 20 °C)	2 500	Lead (μg/l)	10
Hydrogen ion concentration (pH units)[b]	≥6.5 and ≤9.5	Mercury (μg/l)	1
Oxidisability (mg/l O_2)	5	Nickel (μg/l)	20
Aluminium (μg/l)	200	Selenium (μg/l)	10
Ammonium (mg/l)	0.5	Bromate (μg/l)	10
Chloride (mg/l)	250	Fluoride (mg/l)	1.5
Sulfate (mg/l)	250	Cyanide (μg/l)	50
Iron (μg/l)	200	Nitrate (mg/l)	50
Manganese (μg/l)	50	Nitrite (μg/l)	0.5
Sodium (mg/l)	200	Acrylamide (μg/l)	0.1
Total indicative dose (mS/year)	0.1	Benzene (μg/l)	1
Coliform bacteria (number/100 ml)	0	Benzo(a)pyrene (μg/l)	0.01
Microbiological		1,2-Dichlorethane (μg/l)	3
Escherichia coli (number/100 ml)[c]	0	Epichlorohydrin (μg/l)	0.1
Enterococci (number/100 ml)[c]	0	Trichloroethene, tetrachloroethene (μg/l)	10
Chemical		Trihalomethanes – total (μg/l)	100
Antimony (μg/l)	5	Pesticides (μg/l)	0.1
Arsenic (μg/l)	10	Pesticides – total (μg/l)	0.5
Boron (mg/l)	1	Polycyclic aromatic hydrocarbons (μg/l)	0.1
Cadmium (μg/l)	5	Vinylchloride (μg/l)	0.5

[a] Acceptable to consumers and no abnormal change.
[b] May be reduced to pH 4.5 for still water in bottles or containers.
[c] The unit is number/250 ml for water in bottles or containers.

or ultrafiltration. It is also possible to stabilise this water by carbon dioxide to at least pH 6. The requirements for bottled water are the highest for water for to be given to infants.[2] Compared with drinking water, the values of many parameters are several times lower.

7.2.3.2 Food industry requirements

The food industry generally has high requirements for water quality, particularly in microbiological terms. For example, water is one of three basic brewing raw materials. Water hardness and its overall chemical composition is an important factor determining the quality of beer produced in every brewery. Water with a certain higher hardness is more suitable than soft water. For example, Dublin has hard water, which is suitable for producing Guinness, while Munich and Pilsen have softer water, which is particularly suited for producing lager beer. It is known that the calcium content is 14 mg/l in the Pilsner brewery waters, while in Munich brewing waters it reaches 109 mg/l. Both brewing waters also differ in their levels of magnesium (4 and 21 mg/l), bicarbonates (42 and 171 mg/l), sulfates (19 and 79 mg/l) and chlorides (9 and 53 mg/l), respectively. As a result of the water quality (among other reasons), these two beers have a completely different character (organoleptic properties). Beers of lighter colour require lower concentrations of bicarbonates and calcium and higher concentrations of sulfates, while in beers of darker colour it is the opposite; it is related to the buffering capacity of the water, which, together with the pH value,

[2] In many countries there are strict limits on the permissible concentration of nitrate in drinking water and in many surface waters. The limit is 50 mg/l of nitrate in the EU and 44 mg/l in the United States (equivalent to 11.3 and 10 mg of nitrate nitrogen in 1 l, respectively). These limits are in accord with WHO recommendations established in 1970 and reviewed and reconfirmed in 2004 and ensure that drinking water will not cause methaemoglobinaemia, also known as blue-baby syndrome (see Section 6.6.1.2). For example, the permissible concentration of nitrate in bottled water for infants in the Czech Republic is only 15 mg/l.

influences the course of the fermentation. The presence of iron and manganese in large quantities is undesirable (iron concentration is limited to a value of 0.2 mg/l and manganese concentrations have to be even lower).

The dairy industry requires water with a low concentration of magnesium, otherwise the butter has a bitter taste. The iron concentration should not exceed 0.1 mg/l and manganese should be absent. Canning industries, likewise, have similar requirements for the water they use. Higher nitrate concentrations are undesirable in the preservation of vegetables and meat.

The food industry also contributes significantly to the total volume of waste water (mainly from meat processing plants, sugar refineries, breweries and dairies).

7.3 Water in foods

The water content in foods is highly variable. It is related to the chemical composition of the food raw materials (the original animal and plant tissues), the manner of their processing to give the final products and the storage of these products. Water makes up 50–90% by weight of the raw materials of plant and animal origin and of many foods; the rest is called **dry matter**. According to their water content, foods are divided into foods with high, medium and low water contents. The amount of water (water activity) significantly affects the organoleptic characteristics of food (texture, smell, taste and colour) and their shelf life, resistance to microbial attack, enzymatic (biochemical) and non-enzymatic (chemical) reactions that occur during processing and storage.

7.3.1 Water content

The water contents of selected foods are given in Table 7.2.

7.3.1.1 Foods of animal origin

The water content of meat depends on the species (origin) and in particular on the fat content. Owing to its relatively high fat content, pork meat tends to have lower water content (raw pork fat contains about 13% water), while beef has a higher water content. The liver contains 67–72% water. The water content in meat products is highly variable, typically ranging from 30 to 70%. Poultry meat has somewhat lower water content than the meat of other farm animals. Fish generally contain more water. Less oily fish naturally contain more water; fatty fish have a lower water content. For example cod, carp, tuna, mackerel, trout and eel contain 81, 78, 71, 68, 66 and 65% water, respectively.

As the fat content varies, so does the natural water content of milk, within certain limits (87–91%). Soft cottage cheese contains 78% water, while other cheeses can contain less water (52% Camembert, 37–40% Cheddar, Emmental and Roquefort, and 30% Parmesan cheese).

In butter, and therefore in its substitutes (including margarines), the water content varies over a relatively small range. Most butters and margarines contain 16% water. Some special products, such as butter and margarine spreads, and products with reduced fat content, contain around 50% of water; rendered lard contains only traces of water.

The water content of eggs is relatively constant (on average 74%); egg white contains more water (about 88%), while the water content of egg yolk is about 49%.

The amount of water in honey depends upon the type and quality of the honey. Good quality honey essentially has a low water content (generally about 17%). Honey is likely to ferment and lose its freshness if the water content is higher than 19%.

7.3.1.2 Foods of plant origin

The natural water content in the edible part of fruits and fresh fruit juices mainly depends on the type of fruit. Bananas have a relatively

Table 7.2 Water contents in selected foods.

Food	Water content (%)	Food	Water content (%)
Pork meat	30-72	Sugar (saccharose)	0-0,5
Beef meat	35-73	Fruits (juices)	81-94
Chicken	63-77	Vegetables	60-93
Fish	65-81	Potatoes	75-80
Milk (cows')	87-91	Legumes	10-12
Cheeses	30-78	Cereal grains	11-14
Eggs	74	Bread	35-45
Butter, margarines	15-18	Pasta products	9-12
Oils, pork lard	0-0.5	Nuts	3-6
Honey (syrups)	19 (40)	Beer	90-96

low water content (about 76%), while there is more water in pears (about 83%), apples (85%), peaches (89%) and strawberries (90%). Oranges and lemons usually contain 86–87% water. Dried fruits normally contain 12–25% water.

Fresh root vegetables (such as carrots and parsley) are usually about 90% water; cabbage contains about 92% water and lettuce and tomatoes about 95%. There are slightly lower water contents in some bulbous vegetables (89–93% in onion, 83–89% in leek and 61–68% in garlic).

The water content of all cereals is virtually the same as the water content in flours and dry pasta products (9–14%). White bread usually contains 35–36% of water; there is more water in rye bread (38–45%).

7.3.1.3 Other foods

The water content in beverages varies depending on the amount of added sugar and the concentrations of other substances. Fruit juices made from fresh fruit have approximately the same water content as the original fruit. Cola drinks usually contain about 90% water. Vegetable oils and sugar contain only traces of water.

The water content in beer depends on the concentration and strength of the original wort and the degree of fermentation. In other alcoholic beverages, such as liquors, water content depends primarily on the amount of ethanol and on the added sugar.

7.3.2 Changes in water content

Changes to the water contents in foods and food raw materials take place in almost all modes of storage and during all methods of culinary and manufacturing processing. Storage of raw materials and foods in packaging materials that are permeable to water and water vapour leads to a decrease in water content through desiccation. In foods that become moist easily (dehydrated fruit juices and instant beverages such as coffee and tea), the water content increases during storage under humid conditions.

In the manufacture of certain foods such as bread, but also of a wide range of meat, poultry, fish and other products, water is intentionally added to the raw material in a specified amount. The water content also increases significantly during the steeping of pulses, whose chemical components (mainly polysaccharides) are able to bind water. Thermal processing of food by drying, cooking, baking, frying, grilling and roasting usually decreases the water content. In cooked meat, myofibrillar proteins such as actin and myosin contribute significantly to water losses, as they release a certain amount of water during thermal denaturation, which leads to aggregation of these proteins. On the other hand, connective tissue proteins such as collagen bind water.

Losses of water and of water-soluble substances likewise occur during freezing and thawing of foods, depending mainly on the speed of these two operations. If the processes are slow, losses are higher, due to cell damage by large ice crystals, than in the rapid freezing and thawing when the ice crystals are small.

A certain amount of water may also be formed by chemical reactions. Examples are thermal reactions during coffee roasting. The water naturally present in green coffee and water formed by the dehydration of carbohydrates during roasting escapes as steam, along with other volatile products of pyrolytic reactions.

Examples of changes in water (dry matter) content of some common foods of animal and plant origin in different culinary procedures and during industrial processing are shown in Table 7.3.

7.4 Structure

An oxygen atom in the ground state $(1s^2)(2s^2)(2p^4)$ has four valence *p* electrons, two of which are unpaired (Table 7.4). The electronic structure of hydrogen (1*s*) allows connection (hybridisation) of unpaired valence electron of two hydrogen atoms to two unpaired valence electrons *p* of the oxygen atom to four sp^3 orbitals. Two orbitals are bonding (molecular) orbitals, therefore two σ bonds or single covalent bonds O–H are formed and two orbitals are non-bonding orbitals. In the water molecule, H_2O, both elements have the energetically most favourable configuration of the electrons, as in the atoms of the noble gases helium and neon, respectively.

If we imagine the oxygen atom of a water molecule located in the centre of gravity of a regular tetrahedron (the optimal spatial arrangement is tetrahedral from an energy perspective), covalent bonds O–H lead to two tetrahedron vertices and the other two vertices lead to orbitals of the non-bonding electron pairs of oxygen atom (Figure 7.1). The water molecule, like other molecules, is geometrically and physically characterised by the bond (valence) angles of the three connected atoms H–O–H, bond length (distance between the centres of the atoms of oxygen and hydrogen) and energy of the covalent bonds O–H. The bond angle of the O–H covalent bond in a molecule of water is almost identical to the ideal tetrahedral angle (109°28′). The bond angle is 109°6′ in ice, 105° in liquid water and 104°30′ in steam. Differences from the ideal tetrahedral angle are explained by the different repulsive non-bonding forces between the pair of non-bonding and the pair of bonding orbitals (Figure 7.1). The H–O bond length of 0.096 nm is found from the sum of the covalent radii of individual atoms (0.066 nm for oxygen and 0.030 nm for hydrogen). Information about the arrangement of the atom nuclei in space can be obtained from the bond angles and lengths of covalent bonds. Details on the external surface of the molecule result from an imaginary hard sphere called the van der Waals radius, used to model the atom, which is a measure of the maximum distance between the atoms where the van der Waals attractive forces with parts of the water molecule play a role (Figure 7.2). The van der Waals radius is larger than the covalent radius that expresses the spatial requirement of an atom in a covalent bond (0.120 nm for hydrogen and 0.145 nm for oxygen). The energy of covalent O–H bonds in a water molecule (dissociation energy) is about 461 kJ/mol. Intramolecular delocalisation of electrons in covalently bound hydrogen atoms toward the electronegative oxygen atom results in the formation of partial positive charges on both hydrogen atoms (indicated by $\delta+$) and partial negative charge on the oxygen atom ($\delta-$). The water molecule is therefore a polar molecule (it is polarised) and represents an electric (permanent)

Table 7.3 Changes of water content in foods during processing.

Food	Water content (%)	Food	Water content (%)
Pork meat		Sauce (shoyu)	63
Raw	68	Meat (dried)	9
Baked	55	Meat soaked in water for 1 h	65
Fried	53	Meat soaked in water for 10 h	72
Fish (cod)		Meat soaked (10 h) and boiled	79
Raw	81	***Potatoes***	
Preserved	79	Raw	80
Fried	65	Boiled (in skin)	80
Dried (salted)	52	Boiled (peeled)	83
Dehydrated (salted)	12	Flour	8
Milk (3.5% of fat)		French fries (pre-fried)	74
Raw	87	French fries (fried)	55
Pasteurised	87	Crisps (fried)	2
Evaporated (no sweetened)	74	***Apples***	
Condensed (sweetened)	27	After harvest	85
Dried	4	After storage	84
Soybeans		Boiled with sugar (puree)	80
Raw	10	Dried	24
Soaked in water for 1 h	35	Juice	88
Soaked in water for 10 h	60	**Onion**	
Soaked (10 h) and boiled	71	Raw	89
Flour	8	Boiled	92
Curd (tofu)	85	Fried	42
Milk	92	Dried	4

Table 7.4 Some characteristics and electronic structure of the oxygen atom.

Characteristics		Electronic structure in the ground state	
Atomic number	8	1*s*	↑↓
Number of electrons with free spin	2	2*s*	↑↓
Valence	2	2*p*	↑↓ ↑ ↑

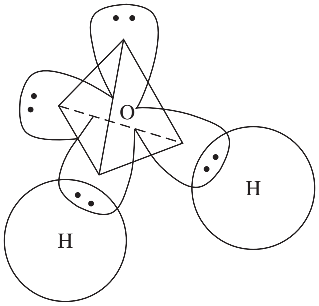


Figure 7.1 Hypothetical structure of a water molecule.

dipole. The measure of the polarity is the dipole moment, which is, compared with other compounds, relatively high: $\mu = 1.85\,\mu_D$ or 1.85×10^{-30} C m, respectively. Electronegativity (the attractive force between the atom and the electron, which is at a distance of the covalent radius) of oxygen is 3.5 eV and that of hydrogen is 2.1 eV (1 eV = 1.6×10^{-19} J). The difference in electronegativities of the two atoms is a measure of bond polarity and expressed as a percentage of its partially ionic character (about 32%).

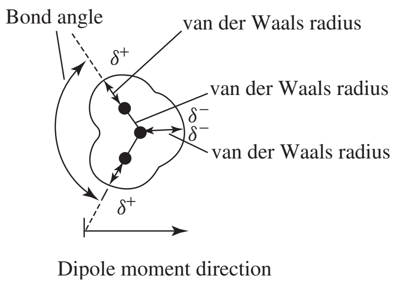


Figure 7.2 Spherical model showing a water molecule with bonding angle, dipole moment and van der Waals radii of hydrogen and oxygen atoms. Zapsalis and Beck, 1985, fig 1. Reproduced by permission of John Wiley and Sons.

Water is a chemical substance that contains 18 different isotopic variants of the H_2O molecule due to the existence of isotopes of hydrogen and oxygen (^{2}H, ^{3}H, ^{17}O and ^{18}O). In addition to undissociated water molecules also present are hydrated hydrogen ions (protons), called oxonium (hydronium) ions (H_3O^+ and trimers $H_9O_3{}^+$, respectively) and hydroxyl ions (HO^-) resulting from dissociation of water and all the variants, which also contain the aforementioned isotopes of hydrogen and oxygen. The natural content of hydrogen and oxygen isotopes is very low (e.g. the amount of ^{2}H is only 0.000 165%); it is therefore sufficient to assume in many situations that the water molecule contains only ^{1}H and ^{16}O isotopes. Significant concentrations of ions in solution, however, exist at extremely low or extremely high pH values, that is, in strongly acidic or strongly alkaline solutions. Biochemical reactions in living systems usually occur in neutral media (pH 6–8); only certain locations of the body may have pH values lower or higher. One ion of H_3O^+ or one ion of HO^- in a solution of pH 7 and at 20 °C corresponds to 7.14×10^8 undissociated water molecules. The concentration of ions H_3O^+ or HO^- is 1×10^{-7} mol/l, while the concentration of undissociated water molecules is 55.6 mol/l.

Food processing often takes place at the natural pH of the food materials of animal and vegetable origin, but the treatment of particular raw materials and the production of certain foods using acidifying or alkalising additives are also common, which strongly influence the course of many biochemical and chemical reactions.

7.5 Properties

Water, unlike related hydrides that contain elements of the sixth group of the Periodic Table (hydrogen sulfide known as sulfan, H_2S, and hydrogen selenide or selan, H_2Se), has fairly unique physical and chemical properties due to mutual interactions of water molecules. The properties of water simultaneously determine many properties of foods. Under normal conditions, water occurs in all three states of matter, whose mutual relations, depending on temperature and pressure, summarises the phase diagram. Water has an anomalous freezing point (ice melting point) and boiling point, high latent heat of fusion (melting or freezing), latent heat of vaporisation (boiling or condensing), specific heat capacity, relative permittivity (dielectric constant), surface tension, viscosity and other physical properties (Table 7.5).

Of note is the dependence of water density on temperature. Water has a maximum density of 0.999 97 kg/l at 3.98 °C and at all other temperatures the density is lower (Figure 7.3). At 0 °C, the density of water is 0.9998 kg/l, but the density of ice at the same temperature is only 0.9168 kg/l. In other words, ice has about a 9% greater volume than water. This increase in volume on converting water into ice (volume expansion) has destructive effects, especially on plant tissues during freezing. The opposite process has the same destructive effect, that is, melting ice (volume contraction) during thawing soft fruits such as strawberries. The destructive effects are also manifested on even more resistant animal tissues. Slow thawing reduces the amount of lost moisture that contains nutritionally valuable substances. The physical data for water, aqueous solutions of various substances and dispersed systems where water is the dispersion medium (solvent), are commonly tabulated and are necessary for engineering calculations in all food technologies.

7.6 Interactions

Water molecules interact with each other, with inorganic substances, as well as with almost any compound or functional group of the organic food constituents, such as proteins, lipids and carbohydrates. All these interactions significantly affect the properties of foods, such as their organoleptic properties,

Table 7.5 Some important physical properties of water compared with ethanol.

Property	Water	Ethanol	Property	Water	Ethanol
Melting point (°C)[a]	0.0	−114.2	Specific heat (kJ/kg)	4.2	2.4
Boiling point (°C)[a]	100.0	78.3	Surface tension (mN/m)[b]	72.8	22.3
Latent heat of fusion (kJ/kg)	333.6	108.0	Dynamic viscosity (mPa/s)[b]	1.005	1.200
Latent heat of boiling (kJ/kg)	2 258	841	Relative permittivity[b]	80.4	24.3

[a]Under normal pressure (101.325 kPa).
[b]At 20 °C.

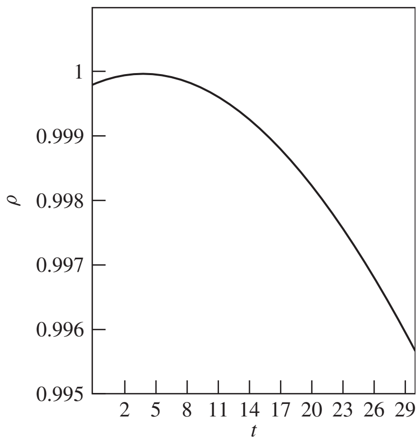


Figure 7.3 Dependence of water density on temperature (ρ = density in kg/l, t = temperature in °C).

Table 7.6 Overview of non-bonded-interactions.

Interactions	Examples
Electrostatic interactions	
Ion–ion	Ions of salts, ionised functional groups of proteins and carbohydrates
Ion–permanent dipole	Ions of salts -water, ionised functional groups of proteins–water, functional groups of carbohydrates–water
Hydrogen bonds	Water–water, functional groups of proteins and carbohydrates
Permanent dipole-permanent dipole	Functional groups of proteins and carbohydrates
Induced dipole-induced dipole	Functional groups of proteins and carbohydrates
Hydrophobic interactions	Hydrophobic functional groups of proteins and lipids

particularly texture, odour and taste. These interactions are also known as **non-covalent interactions** or **non-covalent bonds**, or, generally, as **non-bonded interactions**. They are caused by various attractive and repulsive forces.

Electrostatic attractive and repulsive forces (between ions and dipoles) arising from **electrostatic interactions** and forces that are due to the **hydrophobic interactions** have the greatest significance. These are listed in Table 7.6.

Electrostatic forces between **ions**[3] occur in the interactions of ions formed by dissociation of inorganic salts and in ionic interactions of ionised functional groups of organic substances such as ionised protein functional groups, for example ionised carboxyl groups ($-COO^-$), ionised amino groups ($-NH_3^+$) and ionised functional groups of some polysaccharides ($-COO^-$, OSO_3^-).

An important type of electrostatic interaction are interactions between ions and **dipoles** (permanent dipoles) that occur, for example, during interactions of salt ions with water molecules (the process is called hydration of ions) or during interactions of ionised functional groups of proteins and of some polysaccharides with water. The energy of interaction is several tens of kJ/mol (e.g. 21 kJ/mol in mutual interactions of ionised functional groups of proteins and 50–100 kJ/mol in interactions of monovalent salt ions with water).

Borderline cases of ion–ion and permanent dipole–permanent dipole interactions are the so-called **hydrogen bonds** or **H-bonds**. They occur in compounds containing hydrogen bound to one of the most electronegative elements (such as oxygen, nitrogen or chlorine). This hydrogen has a relatively small but significant affinity for other electronegative atoms. Examples are mutual interactions of water molecules (interaction energy is 17 kJ/mole), interactions of water with polar non-ionised carboxyl groups ($-COOH$) and amino groups ($-NH_2$) of proteins or hydroxyl groups ($-OH$) of carbohydrates and interactions of polar groups (e.g. carboxyl groups, amino groups or other functional groups of proteins). The energy of these interactions is about 12 kJ/mol.

Electrostatic forces between dipoles, known as **dipole interactions** (**dipole–dipole interactions**) are other significant non-bonded interactions. The interactions occur between all types of dipoles, also between permanent dipoles, which are **permanent dipole–permanent dipole interactions**. Examples are interactions between polar functional groups such as a carbonyl group, $-C{=}O$ (a special type of permanent dipole–permanent dipole type of interaction already mentioned are hydrogen bonds). Interactions of the dipole–dipole type are also important for non-polar molecules. At a certain time interval, the distribution of electric charge in non-polar molecules is not uniform. At this given moment, however, some electrons accumulate on one side of the molecule, creating a temporary dipole, which induces dipoles in other molecules. These interactions between induced dipoles are referred to as **induced dipole–induced dipole interactions**. The third and final type of interactions of the dipole–dipole type are interactions between permanent and induced dipoles, known as **permanent dipole–induced dipole interactions**. The sum of the attractive or repulsive forces between molecules or between parts of the same molecule are called van der Waals forces (binding energy is about 1–4 kJ/mol).[4] Special cases are forces between two permanent dipoles, known as

[3] Oppositely charged ions are attracted by the force F (arising from Coulomb's law) acting on a point charge q_1 as a result of the presence of a second point charge q_2: $F = q_1.q_2/4\pi.\varepsilon_0.r^2$, where q_1 and q_2 = electrically charged ions, r = their distance and ε_0 = permittivity of space.

[4] The attractive energy (E) of all types of interactions between dipoles is proportional to r^{-6}, where r is the distance between the centres of interacting molecules ($E = -C.r^{-6}$, C = London constant). With the increasing distance between dipoles (at around 1×10^{-8} m), the so-called retardation effect manifests and the attractive energy is then proportional to r^{-7}.

Keesom forces, forces between two induced dipoles referred to as London dispersion forces and forces between permanent dipoles and induced dipoles called Debye forces.

The hydrophobic interactions occur in non-polar molecules or non-polar functional groups. They are important especially for lipids and proteins. A non-polar substance or non-polar functional group in an aqueous environment is situated in an area surrounded by water molecules associated with each other. In order to create this space, some hydrogen bonds between water molecules must be disrupted, which requires the release of energy. As a result, the free energy of the system decreases and the decrease is compensated by a simultaneous decline in entropy (the system becomes less random, less disorderly and more organised). If two molecules of a non-polar substance are in the same area, the number of broken hydrogen bonds is lower than is the case when non-polar molecules exist separately and are separated by water molecules, because water molecules rearrange around this area so that they create the maximum number of hydrogen bonds. Transfer of non-polar molecules from a non-polar to a polar environment is therefore a spontaneous process, which is accompanied by a negative free energy change. For alkanes this negative free energy change is −10 to −15 kJ/mol and is higher for lipids. Water molecules, which otherwise create hypothetical six-membered structures are partially transformed during the re-organisation into more compact five-membered structures (see Section 7.6.1).

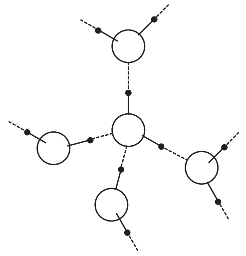


Figure 7.4 Interactions of water molecules (◯ = oxygen atom, • = hydrogen atom, —— = covalent bond, ---- = hydrogen bond).

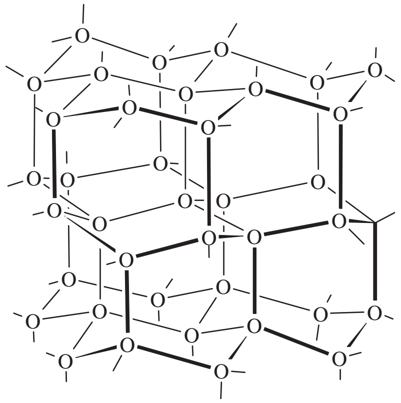


Figure 7.5 Crystal lattice of ice (O = oxygen atoms, hydrogen atoms are not indicated). Fennema, 1985, fig 1.1. Reproduced by permission of Taylor & Francis – Marcel Dekker.

7.6.1 Interactions with water molecules

Water in all states of matter is a highly organised dynamic system. The dipole character of the water molecule allows its association with other water molecules through hydrogen bonds (called hydrogen bridges), which are the interactions of a hydrogen atom covalently bound to the electronegative oxygen atom of one water molecule with the electronegative oxygen atom of another water molecule. In comparison with the length of the covalent bond O–H (0.096 nm), the length of a hydrogen bond (O . . . H distance) is almost doubled and is 0.177 nm; the distance between two adjacent oxygen atoms of water molecules (O . . . O) is 0.276 nm (in ice at 0 °C). The dissociation energy of hydrogen bonds between water molecules is relatively high, at around 5% of the covalent bond O–H dissociation energy (about 25 kJ/mol).

Each water molecule in ordinary ice (called ice I_h, which is the only natural form of ice) associates with four other water molecules. The so-called coordination number (the number of neighbouring molecules located in the immediate vicinity of each water molecule) is therefore 4 (Figure 7.4). Taken together, water molecules in ice create a completely regular spatial association structure that is a hexagonal lattice (Figure 7.5). The crystal lattice structure is not static, since hydrogen bonds in the crystal lattice of ice and of water in liquid and gaseous states, as well as the covalent bond of the water molecules, are only temporary in character. The old links disappear and new links re-form, because hydrogen atoms oscillate, even at temperatures of −20 °C, between two neighbouring oxygen atoms: O–H....O↔O....H–O. The half-life of their duration is only about 10–11 s. Under normal freezing temperatures, only a small number of water molecules are not integrated into the crystal lattice of ice. The number of integrated water molecules in the ice lattice decreases slightly with increasing temperature and increased pressure.

Liquid water at 0 °C has about 90% of the molecules associated by hydrogen bonds as in ice, and in boiling water about 80% of the molecules are still associated. To a small extent, water molecules are associated even in the gas phase. In the liquid state, water molecules are associated in irregular clusters, aggregates of molecules, which are in equilibrium with randomly distributed free molecules. The number of water molecules in clusters is estimated at 200–400 (at 20 °C). Water molecules in clusters are regularly oriented and arranged in a lattice similar to the ice lattice. The duration of clusters is about 1×10^{-8} s.

Liquid water, however, has a greater number of other structures. In addition to the hexagonal lattice found in ice, compact structures, such as the five-membered rings, are also assumed. The consequence of the occurrence of these compact structures is an increase in the coordination number. The coordination number of water in ice at 0 °C is 4; the coordination number of water at 1.5 °C is 4.4 and this continues to rise with increasing temperatures. The average distance between adjacent water molecules also increases with the rising temperature. In ice at 0 °C, the distance of two neighbouring oxygen atoms of water molecules (O . . . O) is equal to 0.276 nm (Figure 7.4), while in water at 1.5 °C this distance is 0.290 nm. The higher water density at 3.98 °C is explained by an

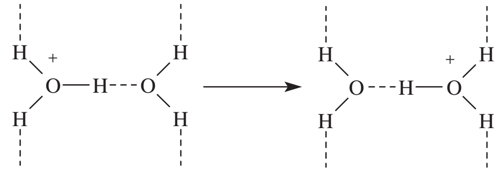


Figure 7.6 Interaction of oxonium ions with water.

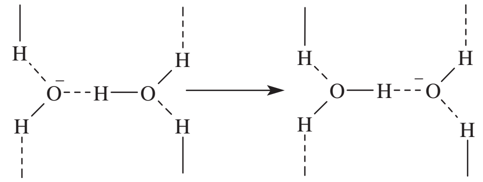


Figure 7.7 Interaction of hydroxyl ions with water.

increase in the value of the coordination number. At temperatures above 3.98 °C, the decrease in water density is due to the increased average distance between adjacent water molecules.

The disturbances in the lattice structure of ice and liquid water also occur in the presence of various non-electrolytes, such as sugars and ions resulting from dissociation of water or electrolytes, for example inorganic acids, hydroxides and salts. Water molecules are associated with protons (oxonium ions) arising by dissociation of water or any acids present. Each molecule of an oxonium ion is associated with three water molecules through very strong hydrogen bonds (dissociation energy is about 100 kJ/mol) and the coordination number is 3. The associated structure is not static, since hydrogen bonds are involved in the transport of protons to other water molecules. The interaction is very fast (about 1×10^{12} per second). Equivalent conductivity and mobility of oxonium ions and of hydroxyl ions is thus very high in comparison with other ions (Figure 7.6). By analogy, hydrogen bonds are also involved in the transport of hydroxyl ions (electrons). Hydroxyl ions are associated with three molecules of water; therefore the coordination number is 3, as in the case of oxonium ions (Figure 7.7).

7.6.2 Interactions with food components

Crucial for the properties of foods are processes taking place at the interface of water and air, water and other liquids and solids, and processes occurring in the aqueous phase of aqueous solutions and dispersions.

7.6.2.1 Interactions with inorganic salts

Inorganic salts are composed of ions, each bound by strong electrostatic attractive forces (interaction of the type ion–ion). This ion (electrovalent) bond generally occurs between two atoms of different electronegativity by transfer of one or more electrons from one atom to another. For example, sodium (Na) is a typical metal (alkali metal) with low electronegativity (0.9 eV) while chlorine is a non-metal (halogen) with high electronegativity (2.9 eV). The positively charged sodium atom with 11 protons and 10 electrons (sodium cation Na^+) arises by transfer of one electron to the chlorine atom, giving rise to a negatively charged chlorine anion Cl^- (with 17 protons and 18 electrons). Both ions gained the electron structure of the rare gases neon and argon, respectively. The crystal lattice of sodium chloride results from the individual cations and anions, cations are surrounded by anions and *vice versa*. Each sodium atom is surrounded by six equidistant chlorine atoms and *vice versa* (sodium and chlorine have the coordination number 6). In the crystalline state, ions have a substantially lower energy than the atoms from which they were formed (Figure 7.8). The result of strong electrostatic attractive forces between the ions is the high melting point (801 °C) and boiling point (1440 °C) of sodium chloride.

Crystals of inorganic salts dissolve well in dipolar water molecules. In order to produce the dissolution of crystals, water molecules break the electrostatic forces binding the ions in the crystal lattice. The energy of the hydrogen bonds between the water molecules (25 kJ/mol) is almost comparable to the energy of ionic bonds, which have electrostatic interactions between the ions (about 21 kJ/mol), and is high enough to overcome the electrostatic attractive forces. Ions released from the crystal lattice (cations and anions) are stabilised in aqueous solution by interactions with water molecules (interaction type ion–dipole). The process is called hydration (generally known as solvation) of ions (Figure 7.9). This leads to association of dispersed ions with several molecules of the dispersion medium (water) and hydrated ions interfere with the regular arrangement

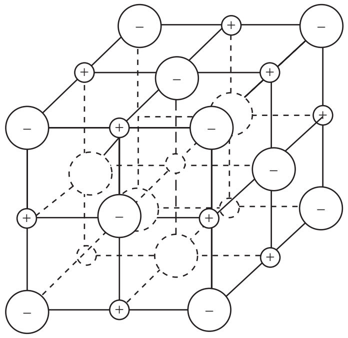


Figure 7.8 Crystal structure of sodium chloride: + = sodium cations, – = chlorine anions.

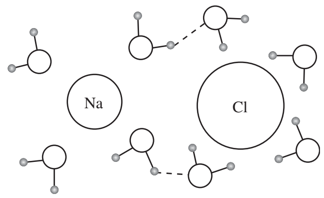


Figure 7.9 Water interactions with sodium and chloride ions. Fennema, 1985, fig 10. Reproduced by permission of Taylor & Francis – Marcel Dekker.

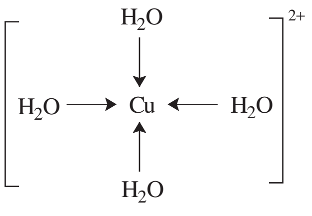
Table 7.7 Hydration of ions (in 0.5–1 mol/l solutions).

Ion	Number of water molecules bound by ion	Ion	Number of water molecules bound by ion
Na^+	8-9	Ca^{2+}	10-12
K^+	4	Cl^-	3
NH_4^+	4	Br^-	2
Mg^{2+}	14	I^-	3-4

of the water molecules (lattice) in their neighbourhood. The result is a somewhat lower water vapour pressure above the solution, lower water activity, higher boiling point and lower freezing point of solution compared with pure water. Also, some properties of ions (such as mobility) are significantly affected by hydration. The degree of hydration of different ions varies depending on the concentration of the ions and other factors (Table 7.7).

Hydration of dissociated ions releases a certain amount of energy called the **hydration energy**. The difference between the crystal lattice energy (energy of the ionic bonds) and hydration energy appears as a **heat of solution**, which is released (positive heat) into the environment or withdrawn (negative heat) from the surroundings. The heat of solution of sodium chloride taken from the surroundings is small (–86 kJ/kg, which corresponds to –5 kJ/mol), but a much higher negative heat of solution (–149 kJ/kg, –27 kJ/mol) arises by dissolving xylitol, which produces a perceived sensation of coolness in the mouth as it comes in contact with the saliva.

A number of metal ions, especially ions of transition metal elements (such as Cu, Zn, Fe, Co and Ni) exist in the form of relatively stable aqua complexes (hydrates) in aqueous solutions at the natural pH values of food materials. Aqua complexes of metal ions are often part of more complicated complexes of mineral compounds with various organic ligands. The binding of water is subject to electrostatic interactions between positive ions and coordinated water molecule dipoles. Generally, the molecules attached to the central ion are called ligands (**7-1**).



7-1, Cu^{2+} cation complex with water

The growth of the pH value leads to hydrolysis of aqua complexes and the formation of less soluble or insoluble hydroxides that have lower biologically availability or are fully available. For example, cupric, ferrous and ferric ions exist in acidic aqueous solutions as very soluble cations $Cu(H_2O)_4^{2+}$, $Fe(H_2O)_4^{2+}$ and $Fe(H_2O)_4^{3+}$, respectively, while in neutral and alkaline media these cations exist as the slightly soluble hydroxides $Cu(OH)_2$, $Fe(OH)_2$ and $Fe(OH)_3$.

7.6.2.2 Interactions with proteins

Proteins are soluble in strongly polar solvents such as water and in aqueous solutions of acids and bases. Certain proteins, known as prolamins, are only soluble in less polar solvents (such as ethanol). The majority of proteins form monodispersed systems (molecules in solution are separated by solvent), but some proteins form oligodispersed systems (see Section 7.8).

The interaction of water with proteins is of paramount importance for maintaining the structure and functional properties of proteins *in vivo*, but also influences texture and other functional properties of foods. Proteins contain essentially three types of functional groups interacting with water:

- **ionised polar groups**, such as amino and carboxyl groups, for example in protein-bound lysine $-(CH_2)_4-NH_3^+$ and glutamic acid $-CH_2-CH_2-COO^-$;
- **electrically neutral polar groups**, such as the ε-amino group of lysine in alkaline media $-(CH_2)_4-NH_2$ and the carboxyl group of glutamic acid in acidic media $-CH_2-CH_2-COOH$ or the hydroxymethyl group of serine $-CH_2-OH$;
- **non-polar groups**, for example alkyl groups of branched aliphatic amino acids valine, leucine and isoleucine, methylthiomethylene group of methionine $-CH_2-S-CH_3$ or aryl groups of aromatic amino acids, such as phenylalanine.

The degree of hydration of protein molecules, which depends mainly on their structure and pH, is expressed as the ratio of bound water weight and protein weight (in g of water bound by 1 g of protein). The degree of hydration is different for each protein, for example about 0.2 in ovalbumin, 0.3 in haemoglobin and up to 0.8 in native β-lactoglobulin.

The pH has a great influence on the ionisation of polar functional groups in the protein-bound amino acids, and thus on the ability of proteins to interact with water (bind water). Ionised functional groups of proteins interact with water in a similar way to salt ions. The ionised basic side chains of lysine and histidine bind about four molecules of water by hydrogen bonds, the acidic side chains of glutamate and aspartate bind about six water molecules, while the neutral carboxyl groups of amino acids (interaction of dipole–dipole type) bind two water molecules of as well as the polar non-ionised side chains of serine and other amino acids.

A thermodynamically disadvantageous situation will occur if a hydrophobic substance or a non-polar functional group of amino acids or proteins is an aqueous environment, because non-polar functional groups associate and the so-called hydrophobic interactions are especially important for maintaining the tertiary structure

of proteins, but also play a role in other situations. Interactions of water with non-polar functional groups of proteins are through the same mechanisms as the interaction of water with lipids. In addition to these interactions, there are also van der Waals interactions.

Only a small proportion of polar groups of native (undenatured) protein located in the hydrophobic area of the molecule are unable to bind water, for steric reasons. Thermal denaturation leads to changes in the higher structures of protein molecules (quaternary, tertiary and secondary) and new, previously inaccessible functional groups, can interact with water and the protein has an increased ability to bind water (30–45%) in the denatured state. If, however, denaturation leads to aggregation of denatured protein molecules, the functional groups of proteins react with themselves, thereby reducing the number of functional groups capable of entering into interactions with water, which decreases the ability of the proteins to bind water.

The behaviour of proteins is significantly affected by the presence of low molecular weight ions (cations and anions of inorganic salts). Many salts (including sodium chloride) increase protein solubility in concentrations up to about 1 mol/l. This phenomenon is explained by the fact that electrically charged dissociated polar groups on the surface of protein molecules bind salt ions via electrostatic forces tighter than water molecules. These ions can bring their own organised clusters of water molecules and the solvated (hydrated) proteins are stabilised. This so-called salting-in effect (see Section 2.4.3.1), in which the solubility of the protein increases slightly, is of great importance in the production of many foods. Water binding of proteins is a particularly important phenomenon in the meat technology sector. For example, if the pH of the meat decreases from 5.0 (which is approximately the value of the isoelectric point of muscle proteins) to about 3.5, the homogenate of muscle proteins may bind double the amount of water than at the initial pH of the meat. Examples are some procedures in cured ham production, when meat salting increases the ability to bind water. The addition of salt solutions for curing, usually by injection or massage, guarantees the desired water and salt contents and makes the meat tenderer. Sodium chloride and sodium chloride ions bind primarily to the myofibrillar proteins actin and myosin. In addition to sodium chloride, sodium and potassium phosphates, citrates and tartrates added to meat and meat products affect muscle pH and increase the meat's water-holding capacity (WHC) and also act as emulsifying agents for processed cheeses (see Section 6.3.4.2.3). In the manufacture of processed cheeses, the added phosphates and polyphosphates act as emulsifiers. They release the casein molecules from micelles and caseins then bind the milk fat or fat added.

At higher salt concentrations (about 1–2 mol/l), the reverse process may occur and many proteins are salted-out or precipitate. The concentration of available water molecules decreases as they are used to solvate (hydrate) the salt ions and the amount of water needed to dissolve the protein is lower. Salts compete for water with the protein. This phenomenon, called the salting-out effect, occurs rarely in food processing except in the surface layers of salted meat and fish and in some speciality meat and fish products with a high salt content.

7.6.2.3 Interactions with lipids

Lipid molecules, such as molecules of triacylglycerols or neutral polar phospholipids, tend to aggregate in an aqueous medium due to hydrophobic interactions of the non-polar parts of molecules. In living organisms, triacylglycerols are stored in the anhydrous form in special cells, adipocytes (fat cells), and act as reservoirs of energy. In hydrothermal processing of food, the fat is released from adipocytes and often accumulates on the water surface as it is adsorbed at the interface with air. Depending on the amount and structure, lipids form a monomolecular film, individual droplets or a continuous layer of fat on the water surface.

Polar lipids, such as glycerophospholipids, sphingolipids and cholesterol, are the structural basis of animal and plant cell biomembranes and cell organelles, such as the nucleus, mitochondria and chloroplasts. Polar lipids are amphiphilic molecules that adsorb on the surface of the water–air interface or water–oil dispersion and stabilise heterogeneous systems such as oil-in-water emulsions (o/w) and water-in-oil (w/o) emulsions. At concentrations higher than their solubility, polar lipids may form macromolecular aggregates called micelles and double films (double layers of molecules known as bimolecular lamellae), respectively. Biomembranes are organised clusters composed mainly of interacting heterolipids and proteins called lipoproteins. They regulate the chemical composition of the intracellular environment by managing the flow of nutrients and metabolic products and facilitate a series of biochemical processes.

7.6.2.4 Interactions with saccharides

The non-bonding interactions of water with monosaccharides, disaccharides, higher oligosaccharides, polysaccharides and other biopolymers containing carbohydrates (such as glycoproteins and glycolipids) take place through hydrogen bonds with electrically neutral hydroxyl groups or possibly with other polar groups. These interactions are of great significance in living systems and also influence the properties of many food dispersed systems.

7.6.2.4.1 Monosaccharides and oligosaccharides

Monosaccharides and disaccharides are generally very soluble in water and many form supersaturated solutions (syrups) during thickening. Similarly to dissolution of other solids, the forces holding the individual molecules in the solid state should be disrupted during the dissolution of carbohydrates. Such forces are cohesive forces of crystals that are, unlike the salt crystals, composed of neutral molecules. Intermolecular attractive forces are due to non-bonding interactions, usually to attractive forces between permanent dipoles. However, other non-bonding interactions, such as van der Waals forces, may also be present.

Water molecules located in the immediate vicinity of dispersed monosaccharide molecules are relatively tightly bound and contribute to the stability of some conformers or anomers. A glucose molecule, for example, is associated with 3.7 molecules of water. The $^4C_1$ glucose conformation (more than 99% of molecules)

is thermodynamically preferred, in which the bulky substituents (secondary hydroxyls) on carbons C-2, C-3 and C-4 are in the equatorial position, therefore far apart. The primary hydroxyl group at C-6, which apparently does not interact with water, and the hemiacetal hydroxyl group at C-1 of the β-anomer are also in equatorial positions. The conformer $^4C_1$ of β-D-glucopyranose thus has four equatorial hydroxyl groups interacting with water (the coordination number, in carbohydrates also called hydration number, is 4), while α-D-glucopyranose has only three equatorial hydroxyl groups interacting with water (hydration number is 3). The average number of equatorial hydroxyl groups of glucose in aqueous solution and the resulting number indicating the number of water molecules located in the immediate vicinity of glucose molecule is: $4 \times 0.67 + 3 \times 0.33 = 3.7$, because D-glucose solution contains approximately 67% β-D-glucopyranose and 33% α-D-glucopyranose in equilibrium.

The distance between the oxygen atoms of hydroxyl groups at C-1 and C-3 (or at C-2 and C-4) of the $^4C_1$ conformer of β-D-glucopyranose is 0.486 nm, and is almost the same as the distance between oxygen atoms in the lattice of liquid water (0.490 nm) and is lying in the same plane (in the ice lattice this distance is only 0.450 nm). By incorporation of glucose molecules into the water lattice, the relative positions of each layer of water lattice molecules virtually do not change and, furthermore, the glucose molecules stabilise the surrounding clusters of water molecules (Figure 7.10). The structural similarity in the interaction with water explains the ability of glucose to slow the formation of ice crystals in frozen confectionery products (similar to the effect of glycerol in mixtures with water that prevent ice from forming) and, particularly, its versatility in biochemical reactions and usability as a building block in living systems.

The range of interactions of other soluble carbohydrates with water also depends on their structure. The organised structure of water molecules is always relatively impaired. A ribose molecule, for example, is associated with 2.5 molecules of water, maltose and saccharose with 5.0–6.6 water molecules. In concentrated solutions of glucose and saccharose, the hydration number is lower, about 2 and 5, respectively. As with electrolyte solutions, solutions of carbohydrates likewise have reduced a water vapour pressure above the solution and lower freezing point and water activity, but the boiling point of the solution increases as compared with pure water (Figure 7.11).

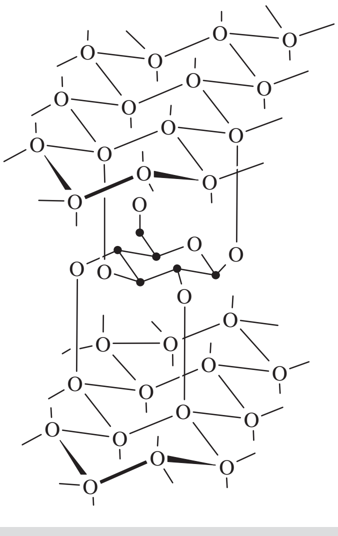


Figure 7.10 β-D-Glucopyranose in an ice lattice: O = oxygen atoms, • = carbon atoms (hydrogen atoms are not marked).

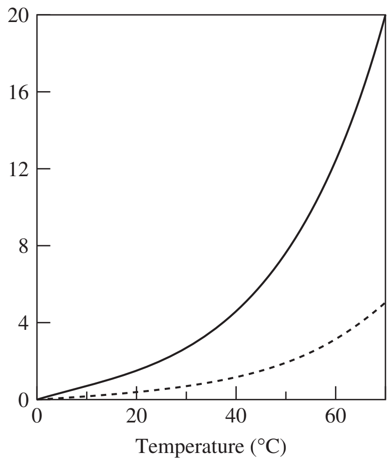


Figure 7.11 Changes of freezing and boiling points of water in comparison with saccharose solutions: c = saccharose concentration (%), —— change of freezing point (°C), – – – change of boiling point (°C).

7.6.2.4.2 Polysaccharides

In the solid state, all polysaccharides contain about 10–20% of water at normal atmospheric humidity. Their molecules or parts of their molecules are involved in different, more or less arranged, structures that create the crystalline and amorphous regions of the molecules. Components of starch (linear amylose with branched amylopectin) are arranged in starch granules, cellulose forms linear microfibrillar structures and agar and carrageenan molecules form double helices. Water is tightly bound in the amorphous regions of molecules by hydrogen bonds through a number of free binding sites (hydroxyl, carboxyl or other polar functional groups) that do not act in interactions with other binding sites of the same molecule (in intramolecular interactions) or with other polymer molecules (intermolecular interactions).

The solubility of polysaccharides and other functional properties depend on their structure. Linear polysaccharides dissolve with difficulty or they are completely insoluble. Such polymers are usually homoglycans with strong intra- and intermolecular non-bonding interactions. As a result of highly organised structures, some parts of the molecules even have crystalline structure and polar groups capable of interaction with water are not available. An example of a polysaccharide insoluble in cold water and soluble in hot water is amylose. Cellulose is insoluble even in hot water, but it is soluble in alkaline solvents.

Linear polysaccharides usually form unstable solutions. During collisions of molecules, some bound water is lost as a result of the

interactions of polysaccharide molecules that form larger aggregates of colloidal dimensions or even coarse dispersion, which may coagulate. These phenomena, illustrating the instability of linear polysaccharides, are well known in the retrogradation of amylose dispersions and starch gels. They also occur in dispersions of arabinoxylans.

Highly branched polysaccharides (such as glycogen, amylopectin and gum arabic) are more soluble than linear polysaccharides, as their mutually interacting functional groups are more distant from each other and thus facilitate hydration. Their solutions have very low viscosity compared with linear polysaccharide solutions of the same molecular weight and the same concentration. This is attributed to the effective volume, the average hypothetical spheres that make up the rotating polysaccharide molecules during thermal motion (Figure 7.12).

The tendency of branched polysaccharides to coagulation is very low. At high concentrations they form sticky pastes, which is probably due to interactions of side chains. After drying, these substances can easily re-hydrate.

Linear branched polysaccharides with a long main chain and a number of short side chains (such as guar and locust gums) combine the properties of linear and highly branched polysaccharides. Since the main chain is long, the viscosity of a solution is higher. Thanks to numerous short side chains, the intermolecular interactions are so strongly attenuated that the solubility and the ability to re-hydrate are high, and concentrated solutions are stable.

Polysaccharides with carboxyl groups hydrate rapidly and form viscous solutions or gels. Examples of these polysaccharides are soluble pectins. Their solubility and ability to form gels is dependent on the degree of polymerisation and the number and distribution of methoxyl groups simultaneously present in the molecule. The solubility generally increases with decreased molecular weight and an increase in the number of methoxyl groups. The pH, temperature and the presence of other substances in solution (saccharose and calcium ions) have a large influence. Other polysaccharides occurring in the form of polyanions (e.g. as esters of sulfuric acid, such as agar and carrageenans) are readily soluble and form highly viscous solutions and gels, such as pectins. The formation of polysaccharide sols and gels is described in Section 7.8.1.

Modified polysaccharides significantly change their properties even at a low level of substitution. The incorporation of acidic groups into molecules of neutral polysaccharides (such as starch phosphates or sodium carboxymethyl cellulose) increases the solubility and viscosity compared with the native polysaccharides. Substitution of neutral functional groups (e.g. in hydroxypropyl starch) increases the solubility, viscosity and stability in solutions. Alkyl groups (in cellulose ethers) facilitate hydration. At a higher degree of substitution, the hydrophobic nature of the polymer increases, along with its solubility in organic solvents.

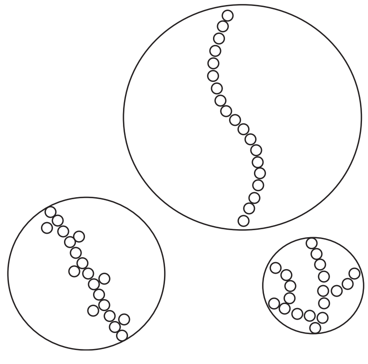


Figure 7.12 Schematic representation of effective volume of linear (A), substituted (B) and branched (C) polysaccharide molecules of the same relative molecular weight (number of monosaccharide units).

7.7 Phase interfaces

The outer boundary of liquid water (of any liquid phase in general), which is in contact with its vapour or the air, is called the **surface**. The surface forming a boundary between two or more separate phases (phase boundary), such as liquid–gas, liquid–solid, gas–solid, or, for immiscible materials, liquid–liquid or solid–solid, is called the **interface**. The surface or interface can be planar or curved. Thermodynamic properties of systems with planar and curved phase interfaces are different.

7.7.1 Surface tension

Water molecules present in the surface or in the interface have different properties in comparison with water molecules located deeper in the homogeneous liquid phase. The resulting attractive forces are directed into the liquid, because interactions with water molecules in the gas phase or with components in the air are insignificant (Figure 7.13). As a result of an imbalance of forces acting on molecules at the interface, a special type of force called **surface tension** is created in the surface.[5] This force acts in the surface plane in a way that resists all efforts to enlarge the surface,

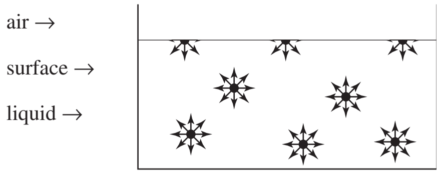


Figure 7.13 Forces acting on molecules of liquids. Zapsalis and Beck, 1985, fig 8.1. Reproduced by permission of John Wiley and Sons.

[5]Surface tension (γ) is defined as the isothermal reversible work that must be made to change the phase boundary by an area unit (A): $dw = \gamma.dA$. When changing the area of the phase boundary with the surrounding medium, not only is the mechanical work exchanged, but also the heat and the total surface energy (σ) is therefore the sum of the work (w) and heat (q) exchanged for a unit change of surface area: $\sigma = w + q$. Constant temperature and pressure leads to the relationship: $\sigma = \gamma - T.d\gamma/dA$, where γ = surface tension, T = absolute

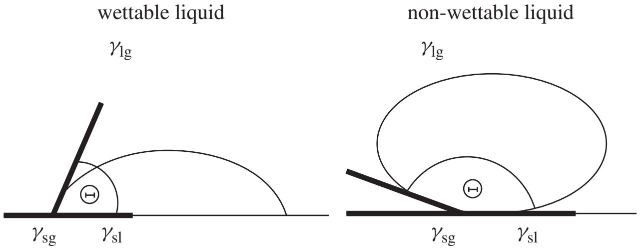


Figure 7.14 Wetting of solids by liquids: γ_{lg} = liquid-air surface tension, γ_{sg} = solid surface energy, γ_{sl} =solid-liquid surface tension, θ = wetting angle.

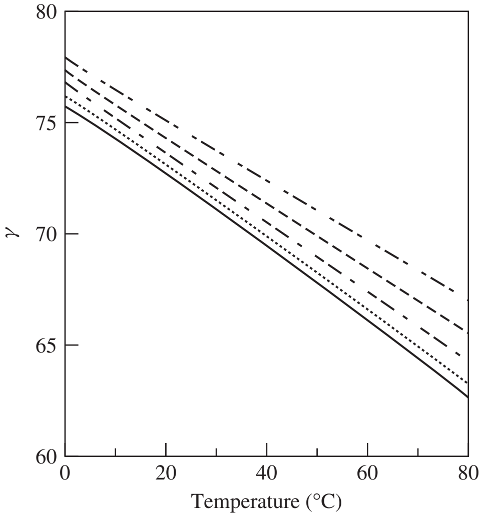


Figure 7.15 Dependence of surface tension of water and aqueous saccharose solutions on temperature and concentration: γ = surface tension (mN/m), temperature (°C), —— 0% (water), ······ = 10%, ·—·—·—· = 20%, —·—·—· = 30%, —··—·· = 40% saccharose solution.

thus tending to reduce the area of the phase boundary (total surface energy) to a minimum. The basic property of the liquid surface is that it tries to occupy the least possible area. The existence of surface tension explains many phenomena taking place at planar interfaces, such as the behaviour of liquids on the surface of other liquids and on the surface of solid materials.

If the liquid is not exposed to external forces (e.g. in a vacuum), it occupies a spherical shape as it gains the smallest surface at the given volume. On the surface of another liquid, it either forms a drop that has a lenticular shape, or can be spread over its surface. The liquid's behaviour at a given temperature depends on the size of the liquid–air surface tension, and on the interfacial liquid–liquid surface tension. On the solid surface the liquid also either spreads out evenly or forms a drop. The shape and size of the drop depend on how the liquid wets the solid surface, which is related to the liquid–air surface tension (γ_{lg}), surface energy of a solid (γ_{sg}), solid–liquid surface tension (γ_{sl}) and temperature (Figure 7.14).

When the surface of a solid substance is in contact with two immiscible liquids, one liquid creates a film between the solid substance and the second liquid. Which of the liquids forms a film depends on both the solid–liquid surface tensions and the liquid–liquid surface tension.[6] The surface tension of pure liquids (such as water) and aqueous solutions (e.g. solutions of saccharose) depends on the temperature: with increasing temperature, surface tension generally decreases (Figure 7.15).

7.7.2 Adsorption

7.7.2.1 Liquid phase-gas phase systems

The surface tension of interfaces between phases where one phase is composed of two or more components (e.g. an interface of an aqueous solution of a solute–air; so-called moving interface) is affected by the solute. For example, the surface tension increases slightly with increasing concentration of polar substances (e.g. salts or sugars, Figure 7.16), and decreases with increasing concentration of substances containing polar and weakly non-polar functional groups (such as lower alcohols and lower carboxylic acids). Surface tension, however, quickly drops to a certain constant value after the addition of **surfactants**. At this value of surface tension, surfactant molecules aggregate to micelles, which remain in the aqueous phase and therefore do not affect the surface tension. Surfactants are substances having in the molecule both hydrophilic (polar) functional groups and strongly lipophilic (hydrophobic, non-polar)

temperature, $d\gamma/dA$ = heat exchanged with the surrounding medium when changing the area of the phase boundary at the given constant temperature.

[6]During contact of more phases, the system is so arranged that the sum of its surface energies is minimal. Liquid A located on the surface of liquid B (three-phase system) will have lenticular shape when $(\gamma_{lg})_B < (\gamma_{lg})_A + (\gamma_{ll})_{AB}$, where γ_{lg} = surface tension of liquids and γ_{ll} = interfacial liquid–liquid tension (with the relevant index A, B or AB), which is always smaller than the surface tension of liquids with higher γ value. If $(\gamma_{lg})_B > (\gamma_{lg})_A + (\gamma_{ll})_{AB}$, liquid A is spread over the surface of liquid B.

In the case of contact of a liquid substance with the surface of a solid substance (three-phase system), the surface energy of the solid compound, the surface energy of a solid (γ_{sg}), interfacial solid–liquid tension (γ_{sl}) and surface tension of a liquid (γ_{lg}) are again applied. The arrangement of the system depends on their values. The liquid is spread on the surface of a solid substance when: $\gamma_{sg} > \gamma_{sl} + \gamma_{lg}$, otherwise it has a drop shape. The relation between surface energy γ_{sg}, surface tension γ_{lg} and interfacial tension γ_{sl} is given by the equation: $\gamma_{sg} = \gamma_{sl} + \gamma_{lg} \cdot \cos\theta$, where θ is the wetting angle (angle of contact). If $\theta < 90°$, the liquid wets the solid surface, if the angle is greater, then the solid surface stays unwetted.

The spreadability of liquid A on the surface of another phase B (liquid or solid substance) can be also expressed using Harkins dimensionless spreading coefficient (S), which is the difference between adhesive and cohesive work: $S = w_a + w_c = \gamma_B - \gamma_A - \gamma_{AB}$. Adhesion work ($w_a$) is the work required for rupture of the column of liquid substance A and the phase B, cohesive work (w_c) is the work required to tear the column of liquid substance A of unit cross section ($w_k = 2\gamma_A$). If $S > 0$, the liquid A spreads on the surface of phase B, otherwise it will create a drop (liquids with a negative value of S are not spreadable). The value of S is affected by temperature.

If in the case of two liquids on a solid surface the following equation is valid, $(\gamma_{sl})_A > (\gamma_{sl})_B + \gamma_{ll}$, the liquid substance B forms a film between the solid substance and the liquid substance A and vice versa in the case where $(\gamma_{sl})_B > (\gamma_{sl})_A + \gamma_{ll}$.

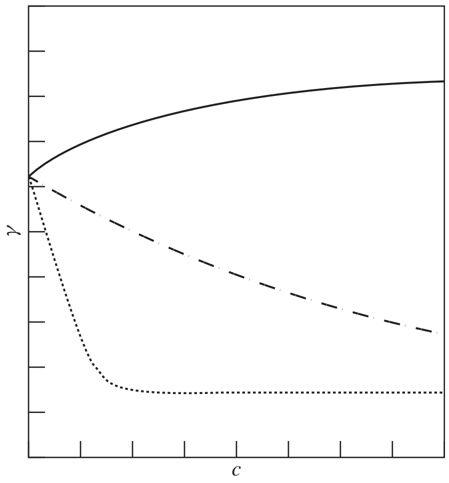


Figure 7.16 Schematic representation of water surface tension depending on the type and amount of dissolved substances: $\gamma =$ surface tension (mN/m), c = concentration, —— = sodium chloride or saccharose, - - - = ethanol or acetic acid (water–air surface tension at 20 °C = 72.8 mN/m), ······· = surfactant.

functional groups. Such substances are called **amphiphilic** or **amphipathic**. In practice, they are used mainly as detergents, emulsifiers, foam-forming substances, solubilisators and wetting agents (Figure 7.16).

The reason that surface tension is influenced by dissolved substances is the fact that the system (aqueous solution of a substance) can bring down its surface energy not only by reducing the phase boundary area as with pure water, but also by changing the concentration of solute at the interphase. Solutes dissolved in a liquid can generally migrate towards the surface or in the opposite direction. The tendency to remove the solute from the solution and concentrate it in the surface is manifested in the case where the mutual interactions of water molecules are stronger than the interactions between water and solute. The surface layer then contains an increased concentration of the solute that reduces the total surface energy (interfacial energy). The phenomenon is called **adsorption**. The substance that is adsorbed is the **adsorbate** and the substance on which another substance adsorbs is the **adsorbent**. Adsorption is a special type of surface aggregation.

Adsorption of liquids and gases can also occur in solid substances. If the liquid or gas does not stay on the surface layer of the solid substance, but penetrates into the interior, the phenomenon is known as absorption (formation of solid solutions). Resolution of both processes (adsorption and absorption) is usually not possible, because it is often a combination of both processes. Therefore, the term often used is sorption.

The forces that cause adsorption at the liquid–gas phase are primarily non-bonding van der Waals forces and hydrophobic interactions. The adsorbed amount increases with the concentration of a substance in the liquid phase and decreases with temperature increase. The action is described by the Gibbs adsorption isotherm.[7]

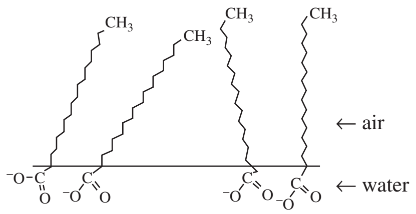


Figure 7.17 Sodium stearate molecules adsorbed on water–air interface at low concentration.

Substances that migrate to the surface and lower the surface tension of liquids are surfactants. The layer in which a significant increase in the concentration of the adsorbing substance occurs, usually has a thickness of only a few molecular diameters, and a film of the surfactant (Figure 7.17) is formed on the water surface.

The formation of films of liquids at surfaces or interfaces is actually a special case of spreading a liquid on the surface of another liquid.[8] If the strong adsorption leads to free spreading of the liquid, the adsorbed substance forms a film one molecule thick, which is called **monomolecular film** (**monomolecular layer**). Monomolecular films also exist in gaseous and solid states. They represent a two-dimensional analogy of the three main three-dimensional states of matter (gaseous, liquid and solid). Molecules of the substance in the surface film are oriented by their polar parts to the liquid (water) and by their non-polar parts to the less polar environment, which is air (Figure 7.18). Monomolecular films have a measurable effect on the mechanical, optical and electrical properties of surfaces and interfaces. The external force, which the film causes during compression, is usually called **surface pressure**.[9] If there is not enough space on the surface, the substance

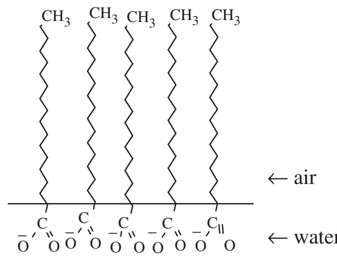


Figure 7.18 Sodium stearate molecules adsorbed in monomolecular film.

[7]Relation between the surface excess of a component (Γ) that migrates to the liquid surface and the change of its surface tension with concentration of adsorbate ($d\gamma/dc$) is expressed by the Gibbs adsorption isotherm, which is valid for ideal adsorption: $-\Gamma = c/RT.d\gamma/dc$, where $c =$ concentration of solute, $R =$ universal gas constant, $T =$ (absolute) thermodynamic temperature (K). In addition to adsorption other phenomena also often occur.

[8]If $(\gamma_{lg})_B > (\gamma_{lg})_A + (\gamma_{ll})_{AB}$, liquid A (surfactant) is spread on the surface of the liquid B (water). Likewise, under certain circumstances, on the surface of the water can be spread a small amount of insoluble and non-volatile substance applied appropriately to the surface.

[9]The surface pressure (π) and surface tension are related by the equation: $\pi = \pi = \gamma - \gamma$ ($\gamma =$ surface tension of pure liquid, $\gamma =$ surface tension of liquid covered with a film). The relationship between surface pressure (π) and surface area (A), which falls on one molecule of a substance is: $\pi = k.T/A = k_B.T/(A-a)$,

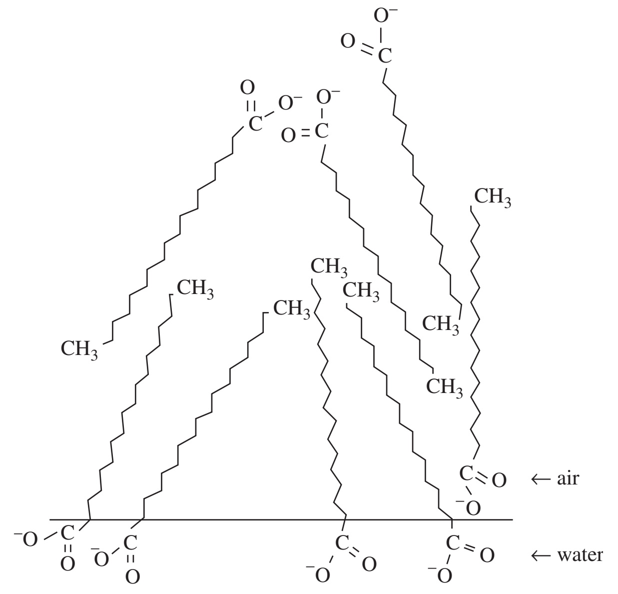


Figure 7.19 Sodium stearate molecules adsorbed on water–air interface at high concentration (multimolecular film).

partly creates a monomolecular film and the excess remains in the form of drops, which are in equilibrium with the film. At higher concentrations of a substance, a multi-molecular film or even a layer of a substance is formed (Figure 7.19).

Substances strongly interacting with water (such as hydrated ions) migrate, unlike surfactants, away from the surface of the liquid as they increase the total surface energy and therefore their interphase concentration decreases.

7.7.2.2 Liquid phase–liquid phase systems

Adsorption at the interface of two liquid phases has the same characteristics as the adsorption at the interface of a liquid phase–gas phase, since it is again a moving interface. Adsorption can be therefore characterised in the same way as adsorption systems in a liquid phase–gas phase (using Gibbs isotherm).

In practice, the most important interfaces are those of the polar phase (water) with a less polar or non-polar phase and a water-immiscible liquid phase (oil). In these systems (e.g. in emulsions) surfactants are employed, whose polar functional groups interact with water molecules (solvation) and the non-polar functional groups interact with the particles of the non-polar phase.

7.7.2.3 Solid phase–gas phase systems

Liquid–gas or liquid–liquid phase interfaces are plain or concave or convex, but the surfaces of solids are almost always non-homogeneous and contain a variety of edges, notches, pores and capillaries. The forces that cause adsorption at the solid–gas interface are, again, primarily non-bonding interactions of van der Waals forces and hydrophobic interactions. This so-called **physical adsorption** takes place on the entire surface, but there is no monomolecular layer, but multiple layers of the adsorbing substance arise. Physical adsorption is very fast, almost instantaneous. The porous solids in the pores can also contain liquid phase. This phenomenon is called **capillary condensation**.

At the same time, in the active centres in solids interactions between solid and liquid phases occur, having the character of chemical bonds. This type of adsorption is therefore called **chemisorption**. The chemisorption process is slower than physical adsorption, as it is preceded by diffusion of the substance to the outer surface of the adsorbent and diffusion through the adsorbent pores to the surface (to the active centres).[10]

Solid–gas phase interfaces are therefore described by other means, such as by the Freundlich, Langmuir and BET isotherm.[11] Heat of adsorption during the physical adsorption is of the same order as heat of condensation. Adsorption heat during chemisorption is of the same order as the reaction heat.[12]

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where k_B = Boltzmann constant ($k_B = R/N_A$, R = gas constant, N_A = Avogadro constant), T = absolute temperature, a = area occupied by one molecule in a saturated monomolecular film. The value of (a) is practically the same for a number of organic compounds (around 20×10^{-20} m^2, that is 0.2 nm^2).

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[10] Chemisorption rate constant $k = A.\exp(-E/RT)$, where A = frequency factor, E = activation energy of adsorption of a substance, R = gas constant and T = absolute temperature.

[11] The Gibbs adsorption isotherm is valid generally for each phase boundary, but is used mainly for mobile phase interfaces (gas–liquid and liquid–liquid systems). Solid phase–gas phase systems do not use the variable Γ (as surface energy is not constant), but the amount of gas adsorbed (a), which depends on temperature and pressure. It represents the amount of adsorbate to the amount of solid adsorbent and is expressed mostly in moles or mass units. Adsorption can then be characterised, for example, by an empirical Freundlich isotherm: $a = k.p^{1/n}$ (or in linearised form: $\ln a = \ln k + 1/n.\ln p$), where $a = x/m$, x = amount of adsorbate, m = amount of adsorbent, p = equilibrium pressure, k and n = constants for given adsorbate and adsorbent at a particular temperature.
The Langmuir adsorption isotherm is a good description of processes based on chemisorptions: $a = a_m.b.p/(1 + b.p)$, in linearised form: $p/a = 1/a_m.b + p/a_m$, where b = constant, m = constant (or the amount adsorbed when the adsorbent surface is completely covered with a monomolecular layer of adsorbed substance).
The BET isotherm (named after the authors Brunauer, Emmett and Teller) provides multilayer adsorption, when other layers are created on the first monomolecular layer of particles due to intermolecular attractive forces between particles in the monomolecular layer and particles of the gas phase. A BET isotherm has the form: $a = a_m.C.p/(p^0-p).[1 + (C-1).(p/p^0)]$, where p^0 = saturated vapour pressure, $C = \exp q_k\text{-}q/R.T$, q_k = heat of condensation and q = heat of adsorption. If $C < 1$, the first process is adsorption on the monomolecular layer followed by adsorption on the second and other layers. If $C > 1$, multilayer clusters form instead of the monomolecular layer.

[12] Heat of adsorption is expressed as a differential heat of adsorption (q) or as an integral heat of adsorption (ΔH). Integral heat of adsorption can be determined by calorimetry, differential heat of adsorption (q) is calculated from the measured isosteres: $[\mathrm{dln}\, p/\mathrm{d}T]_a = -q/RT^2$. Differential heat of adsorption heat (q) is defined as heat that is released upon adsorption of 1 mole of gaseous substances in a quantity of the sorbent that the adsorbed amount (a) per unit mass of adsorbent remains constant. Integral heat of adsorption (ΔH) is the heat that is released upon adsorption of 1 mole of a substance in a quantity of the sorbent that the adsorption is just equal to the value of adsorbed amount (a):

$$\Delta H = 1/a \int_0^a q.\mathrm{d}a$$

7.7.2.4 Solid phase–liquid phase systems

Adsorption at the solid–liquid interface is generally similar to the adsorption at the solid phase–gaseous phase interface. Theoretical modelling of the adsorption process is more difficult because, in addition to adsorbing dissolved substances, solvent is present (e.g. water), the molecules of which can also adsorb, and interactions of adsorbed molecules with molecules of the solvent may occur. Molecular adsorptions, when molecules of a substance are adsorbed, and ion adsorptions, in which ions of a substance are adsorbed, can similarly take place. Solid phase–liquid phase interfaces are usually described by empirical equations and theoretically derived equations of the Freundlich and Langmuir type.

7.7.3 Capillary phenomena

The curvature of the surface of the phase boundary creates a number of important so-called **capillary phenomena**. A large curvature of the surface of the phase boundary is characteristic of systems that contain small particles (highly dispersed systems). The result is, for example, an increased pressure (so-called Laplace pressure) inside the droplet of a certain radius due to the change in its size (radius). Another consequence is that the liquid substance in a capillary tube, with one end submerged in a container of liquid, rises so high that the hydrostatic pressure of its column is equal to the Laplace pressure.[13] In other words, adhesion forces between the fluid and the solid inner wall pulls the liquid column until there is a sufficient mass of liquid for gravitational forces to overcome these intermolecular forces.

The free surface of a liquid, the meniscus can be flat or it can have a convex or concave shape, depending on the solid and liquid surface. The forces acting on the molecule of a liquid at the free surface are: the weight of the molecule, the force of adhesion and the force of cohesion that act in various directions, therefore differences in the shape of the meniscus may occur. Pressure on the concave side of the curved surface depends on the radius of curvature and surface tension of a liquid. If the liquid is in a capillary, the radius of curvature of its surface is small and the pressure difference on both sides of the meniscus is large. If it is a liquid substance that wets the capillary walls, the liquid creates a meniscus whose curvature depends on the radius of the capillary and the wetting angle. The meniscus can be concave upwards, and the capillary liquid level rises above the liquid that surrounds it. This phenomenon is called capillary elevation. If the liquid substance does not wet the capillary wall, it creates a meniscus that is convex upwards, and its level is below the surface of the liquid surrounding it. This phenomenon is called capillary depression (Figure 7.20).

Capillary rise of solutions in the pores is of paramount importance for the water supply of plant tissues, and also for the wetting and drying of foods. Capillary phenomena also explain the hysteresis that occurs during adsorption and desorption of water in food. The so-called capillary condensation occurs during adsorption in solid phase–gas phase or solid phase–liquid phase systems.[14]

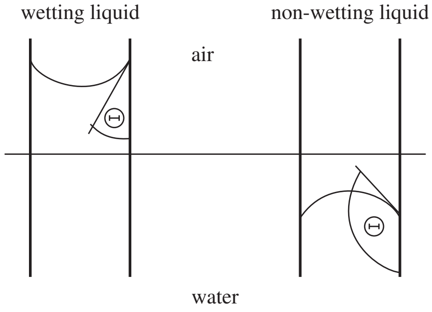

Figure 7.20 Capillary wetting by liquids (θ = contact angle).

7.8 Food dispersed systems

Dispersed systems (dispersions) are mixtures of at least two substances, one of which (dispersed phase or internal phase) is often discontinuous and is distributed in the form of particles throughout another substance (continuous phase also known as external phase or dispersion medium). Dispersions are all systems, which consist of at least two types of particles (molecules of dispersed phase and molecules of continuous phase) or of two different phases (solid, liquid or gaseous).

The dispersion medium of all living cells is water. If liquid water is in contact with its vapour or air, or with another liquid (with which it is not well mixed) or solid, it forms a two-phase or polyphase system. Water is also a dispersion medium for low and high molecular weight chemical components found in food. These components are

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[13]Relationship is given by the equation: $\Delta p = 2\gamma/r = \rho.g.h = 2\gamma.\cos\theta/R$, where Δp = Laplace pressure, the difference between pressure of the liquid and pressure of the surrounding area, r = radius of curvature = $R/\cos\theta$ (R = radius of capillary, θ = contact angle), $\rho.g.h$ = hydrostatic pressure, h = height of liquid column. The difference of the liquid level in the vessel and capillary $\Delta h = 2\gamma_1/\rho.g.l$, where γ_1 = surface tension of liquid, ρ = its density, g = gravitational acceleration and r = capillary radius.

[14]Vapour pressure over the concave meniscus is lower than over the plane surface. Therefore, the vapour pressure (p) above the liquid that filled the narrow pores of the adsorbent is lower than the vapour pressure of saturated vapour above the liquid with a flat surface (p_0), which is given by the Kelvin equation: $R.T.\ln p/p_0 = -2\gamma.v/r'$, where R = gas constant, T = absolute temperature, γ = surface pressure of liquid, V = molar volume of liquid condensed in the pores, r' = radius of the meniscus ($r' = r/\cos\theta$, where r = pore radius and θ = wetting angle).
The Kelvin equation also shows that the penetration of water into the large pores of relatively large diameter r_2 located inside the food (during water adsorption) that are attached to the surface by a capillary of diameter r_1 depends on the pore radius: $p = p_0 \cdot \exp(-2\gamma.v/r_2.R.T)$. The immediate emptying of pores occurs when the vapour pressure during desorption is equal to $p_d = p_0 \cdot \exp(-2\gamma.v/r_1.R.T)$. According to the theory of the so-called open pores, the mechanisms of adsorption and desorption are different. Adsorption of water within the capillaries takes place on the inner wall where the water is adsorbed in a thin film of thickness D. The water vapour pressure during adsorption (p_a) is then described by the Cohan equation: $p_a = p_0 \cdot \exp[-\gamma.v/(r_1 - D).R.T]$. Desorption (liquid fills the whole capillary and $\cos\theta = 1$) is described by the Kelvin equation. Water vapour pressure during the desorption is $p_d = p_0.\exp(-2\gamma.v/r'.R.T)$. In cases where $r_1 > 2D$, is $p_a > p_d$ and hysteresis occurs, when $r_1 = 2D$ hysteresis does not occur.

water-dispersed (scattered) in the form of ions, neutral molecules or different particles that form the dispersed phase.

Dispersed systems are common aqueous solutions of low molecular weight compounds, salts, amino acids, mono- and oligosaccharides (such as sodium chloride, glycine and saccharose), as they contain at least two types of particles (molecules of the water-dispersion medium and the ions or molecules of the substance-dispersed phase). These systems are **monodispersed systems** because their components contain particles of about the same size with a certain relative molecular weight (in daltons, Da) or molar weight (in kilogram per mole, kg/mol, or gram per mole, g/mol).

Dispersed systems are also solutions of macromolecular compounds, proteins and polysaccharides, which are normally mixtures of polymer homologues containing particles of different sizes (often monomers, dimers, higher oligomers to polymers of the highest molecular weights). They are therefore **polydispersed systems** that do not have exact relative molecular weight. For their characterisation, the mean relative molecular weight or medium polymerisation degree is used (number of bound monomer units). An important point for the characterisation of polydispersed substances is the distribution curve, which indicates the frequency at which the individual particles occur in the dispersed system, depending on their molecular weight. In some polymers, the particles differ not only in size (such as fructans), but also in structure as some of them can be branched in a different way (arabinoxylans) or can have different chemical compositions (alginates). In such cases, their distribution is known as chemical distribution.

7.8.1 Classification of dispersed systems

Dispersed systems are classified according to the number of phases, the shape of dispersed particles, particle size, state (gaseous, liquid or solid) of dispersion medium and state of dispersed phase. In practice, foods can contain all types of dispersed systems.

Depending on the number of phases, dispersed systems are divided into:

- **homogeneous dispersed systems**, where the dispersion medium and dispersed phase form one phase;

- **heterogeneous dispersed systems** in which the dispersion medium and dispersed phase are two different phases separated by a phase interface; if the dispersed phase particles are smaller, the phase interface area is larger and affects the system behaviour more.

Homogeneous dispersed systems arise spontaneously and are stable (such as true solutions). Heterogeneous dispersed systems do not arise spontaneously, but are prepared artificially. They contain a dispersed phase not associated with molecules of solvent (usually water), and they are non-equilibrium dispersed systems, therefore they are unstable and spontaneously disappear.

According to the shape of the dispersed phase particles, dispersed systems are divided into:

- **globular dispersions** (all three dimensions are of the same order, they are isometric);

- **laminar dispersions** (one dimension is smaller by one order of magnitude);

- **fibrillar dispersions** (two dimensions are smaller by one order of magnitude).

Depending on the size of the dispersed phase particles (expressed as a radius, volume or weight), which corresponds to the degree of dispersion of the dispersed phase in the dispersion medium, the dispersed systems are divided into three groups (real dispersed systems, however, form a continuous series without distinct boundaries):

- **molecular dispersions** are characterised by a high degree of dispersability; the dispersed phase particles (molecules or ions) have a size less than 1 nm, are not observable by electron microscopy, easily diffuse and penetrate through membranes (dialysis), show very intense thermal motion (Brownian motion), lead to high osmotic pressures, systems are homogeneous and always form true solutions that arise spontaneously and are stable;

- **colloids** (colloidal dispersions or colloidal systems) are characterised by moderate dispersion; the size of colloidal particles is 1–1000 nm, particles can be observed by electron microscopy, diffuse slowly, penetrate some membranes, perform intense Brownian motion, lead to a measurable osmotic pressure; colloids are often allocated into three categories: **associative colloids** or **micellar colloids** (that form a transition between molecular dispersions and colloids), **lyophilic colloids** (that are homogeneous and form colloidal solutions) and **lyophobic colloids** (that are heterogeneous and consist of two phases or form multiphase dispersed systems);

- **coarse dispersions** (suspensions) are heterogeneous dispersed systems, in which the dispersed phase particles are larger than 1000 nm; systems with a particle size within 1000–50 000 nm are called **micro dispersed systems**; systems with a larger particle size (>50 000 nm) are **macro dispersed systems**, particles of coarse dispersions are observable by optical microscope and are characterised by relatively fast sedimentation due to gravity or other forces, perform Brownian motion only to a maximum particle size of 4000 nm, do not cause osmotic pressure and are always heterogeneous and unstable.

Dispersed systems are often classified according to the state of the dispersion medium and the state of the dispersed phase (gas, liquid and solid) into nine types (Table 7.8). The most common dispersion systems are sols, gels, emulsions and foams.

7.8.1.1 Molecular dispersions

Molecular dispersions are true solutions. They arise, for example, by dissolution of inorganic salts or low molecular weight organic compounds, such as monosaccharides or amino acids. The dispersed phase is dispersed into ions (e.g. sodium chloride) or molecules (such as glucose or air), which are of comparable size to the size of the molecules of the dispersion medium (water). True solutions are

Table 7.8 Types of colloids.

Dispersed phase	Dispersion medium	Name of dispersion	Examples
Solid	Solid	Solid solution or solid sol or solid mixture	Iodised salt (mixture of NaCl and NaI)
Solid	Liquid	Sol (lyosol), true or colloidal solution, suspension	Whey
Solid	Gas	Aerosol (smoke)	Plant protection products (fumigants)
Liquid	Solid	Gel or solid emulsion	Cosmetic products
Liquid	Liquid	Emulsion or solution of liquid in liquid	Milk, mayonnaise, butter, margarine
Liquid	Gas	Aerosol (fog)	Plant protection products
Gas	Solid	Solid foam	Ice cream, foam sweets
Gas	Liquid	Foam or solution of gas in liquid	Whipped cream
Gas	Gas	Mixture of gases	Air

usually kinetically stable (resistant to the effects of the gravitational field) and aggregation (still have the same degree of dispersion).

7.8.1.2 Colloidal dispersions

Colloidal dispersions (colloids) are basically formed in two ways:

- The first possibility is spontaneous reversible association (aggregation) of some low molecular weight unstable organic and inorganic substances in true solutions, as a consequence of attractive forces **associative colloids** or **micellar colloids** appear. An example of micellar colloids is casein micelles in milk.

- The second possibility is dissolution of high molecular weight substances, such as certain proteins, polysaccharides (including nucleic acids or synthetic polymers), which leads to homogeneous dispersion systems called **lyophilic colloids** that arise in various solvents. If the solvent is water then **hydrophilic colloids** are formed. Colloids containing individual molecules of dispersed phase (substance) are sometimes called **molecular dispersions**. Heterogeneous dispersed systems called **lyophobic colloids** or **hydrophobic colloids** are prepared by physical or chemical methods, such as by dispersion of non-polar substances in water. They resemble coarse dispersions and are therefore also called **phase colloids** or **dispersoids**. The common feature of all colloids is their large surface area, which is greater in smaller particles and surface phenomena therefore often determine the properties of colloidal dispersions.

7.8.1.2.1 Micellar colloids

The formation of micellar colloids is mainly a result of hydrophobic interactions (the corresponding entropy change is about 13 kJ/mol) and van der Waals attractive forces between non-polar parts of the molecules. The resulting spherical associates (aggregates) of colloidal dimensions are called micelles. The number of molecules in the micelles depends on the properties of the substances that form the micelles, their concentration and temperature. Micelles contain tens of thousands of molecules of the constituent. Micellar colloids are usually globular or laminar dispersed systems. Micelle formation is typical for all amphiphilic substances. Spherical and ellipsoidal micelles typically consist of fatty acids, glycerophospholipids and also proteins that contain hydrophobic and hydrophilic portion in the molecule (e.g. caseins in milk). Association of molecules occurs only at a concentration that is characteristic of the material, called the critical micelle concentration (dispersions of lower concentration provide true solutions or colloids). For amphiphilic constituents with one hydrophobic end, such as soaps (e.g. salts of stearic acid) and other surfactants (such as dodecyl sulfate), the critical micelle concentration is approximately 1 mmol/l, for amphiphilic substances with two hydrophobic ends (such as glycerophospholipids), the critical micelle concentration is generally 1 mol/l.

Classical examples of micellar colloids are dilute aqueous dispersions of fatty acids (their salts are accordingly known as soaps) and aqueous dispersions of other detergents. At a concentration below the critical micelle concentration, true solutions are formed (Figure 7.21), while at the critical micelle concentration and higher, micellar colloids are formed (Figure 7.22). The interiors of the micelles are filled with concentrically oriented hydrophobic groups, while on the surface are hydrophilic ionised polar groups interacting with counter ions (e.g. Na^+ cations) from the environment. These micelles are called ionic micelles. The arrangement into micelles is advantageous since it eliminates undesirable contact of water with the hydrophobic parts of amphion molecules and allows the polar groups contact with water.

Concentrated solutions of soaps also yield laminar micelles that are formed by non-dissociated molecules. Their shape is similar to the shape of glycerophospholipid micelles. Glycerophospholipids (or sphingolipids) contain two non-polar chains in the molecule and, for steric reasons, tend to form large ellipsoidal micelles, which

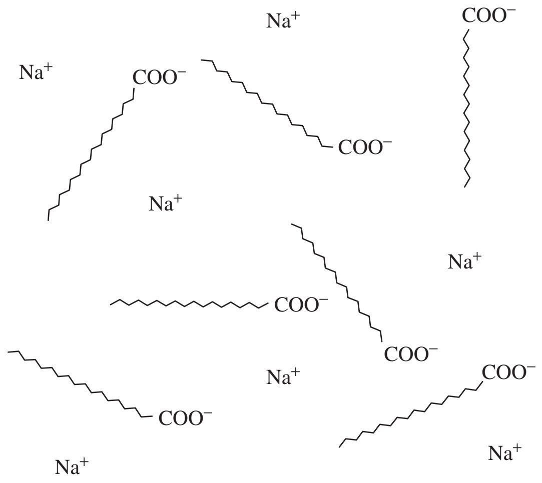


Figure 7.21 True solution of dissociated sodium stearate molecules.

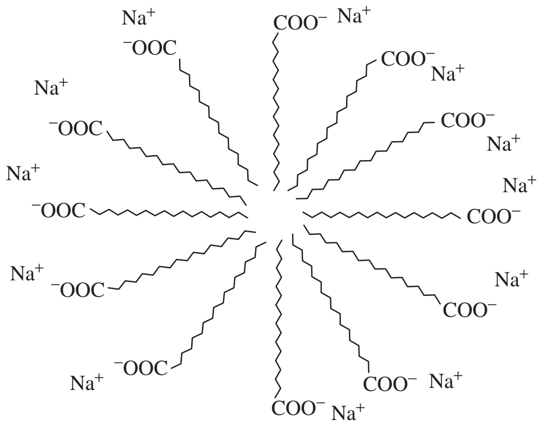


Figure 7.22 Micelle of dissociated sodium stearate molecules.

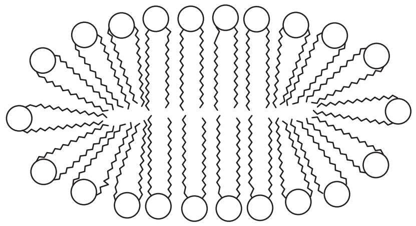


Figure 7.23 Double layers of phospholipids.

are actually a double layer of molecules. They are called bimolecular sheets or bimolecular lamellae or binary films (Figure 7.23). Milk is also a complex micellar colloid containing clusters of casein molecules called submicelles, which are associated to micelles by citric acid molecules and calcium ions.

7.8.1.3 Coarse dispersions

Coarse dispersions contain particles that are not individual chemicals (e.g. particles of food dressings containing particles of vegetables and spices), and are usually strongly polydispersed. The representation of a particle size in a polydispersed system is expressed by various distribution functions, for example, the mean radius of the particles and other particle characteristics can be determined.

7.8.2 Stability of dispersed systems

Colloidal dispersed systems are characterised by high surface energy and high curvature of the surface particles. From a thermodynamic point of view, these systems have low aggregate and kinetic stability. Their aggregate instability manifests by two spontaneous processes:

- slow dissolution of small dispersed particles, while the growth of larger particles is not usually significant (the speed of this process can be regulated in liquid dispersion media by changes in temperature or composition of the dispersion medium);
- **coagulation**, which is clustering of dispersed particles into larger aggregates associated by adhesion forces of the particle surfaces; the process is also called **flocculation**; when aggregation leads to coarse dispersions, sedimentation of aggregates may occur; coagulation, which is often one of the most important processes, can be caused by various interventions.

7.8.3 Important dispersed systems

7.8.3.1 Sols

Dispersions of solids (dispersed phases) in liquids (dispersion media) are called **sols**, or in general **lyosols**. Heterogeneous dispersed systems with only weak interactions between the dispersed phase and dispersion medium are called **lyophobic sols**. Homogeneous dispersed systems with strong interactions between the dispersed phase and the dispersion medium are called **lyophilic sols**. The interaction of particles of lyophilic sols with the dispersion medium leads to **solvation**. If the dispersion medium is water, the preferred terms are **hydrosol**, **hydrophobic sol**, **hydrophilic sol** and **hydration**.

Hydrophobic sols are, like all lyophobic colloids, heterogeneous. The dispersed phase and dispersion medium represent two different phases separated by the phase interface. Hydrophobic sols arise spontaneously, but are unstable. They are difficult to prepare (e.g. by mechanical agitation of solid particles, stirring or condensation of small particles to micelles of colloidal dimensions) and require the presence of surfactants. They are also more stable in the presence of hydrophilic sols.

Hydrophilic sols are homogeneous colloids that give colloidal solutions. They can form spontaneously and are relatively stable. Colloidal solutions of proteins and polysaccharides are typical hydrophilic sols. Hydrophilic sols are prepared by dissolving the solids in water. For example, proteins and polysaccharides are

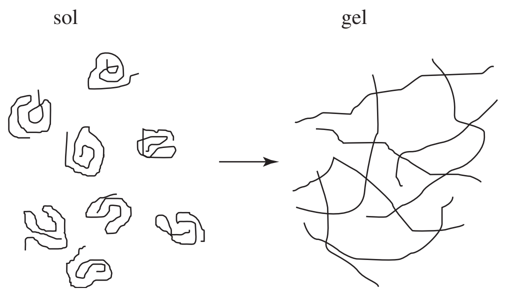


Figure 7.24 Scheme of gelatine gel formation.

suspended in water and the suspension usually requires heating. The sol, the dispersion of the solids (dispersed phase) in an aqueous dispersion medium, forms when there are only weak interactions between the macromolecules. In gelatine sol, individual protein molecules are compact, in the shape of randomly coiled globules. A sol behaves as a liquid (Figure 7.24).

Dissolution of proteins and polysaccharides is not a simple process. The solids often absorb a certain proportion of the liquid (water) with which they are in contact, without losing their shape, which is the most important property of these solids. The process is called **imbibition**. Imbibition occurs, for example, in starch granules at temperatures lower than the gelatinisation temperature (see Section 4.5.6.1.3). Solids can take up even more fluid, which increases their volume and results in **swelling**. The particle volume during swelling does not increase proportionally to the volume of absorbed fluid (water), but the sum of volumes of the substance and absorbed water is always less than the volume of swollen product, which therefore has a higher density than the original material and subjects its surroundings to a certain pressure, **swelling pressure**. Swelling is also accompanied by release of heat and by changes to the mechanical properties, primarily in increased strength and elasticity.

Depending on the mechanism of swelling, the following categories are recognised:

- Capillary swelling, which occurs mainly with the fibrous structure of organic compounds, such as many polysaccharides; solvent (water) penetrates between the individual molecules of the constituents that are solvated and the particles move away from each other during swelling, which increases the degree of dispersal.

- Molecular swelling, during which the particles of the constituent material swell, their volume grows and the degree of dispersal decreases.

According to the degree of swelling, the process can be:

- limited

- unlimited.

Examples of limited swelling are gelatine and agar in warm water (e.g. at 25 °C). Examples of unlimited swelling are gum arabic or soluble starch, which swell in water at any temperature. Gelatine and agar show unlimited swelling in hot water (e.g. at 95 °C).

In gelatine and soluble polysaccharides (such as agar) in an aqueous environment, water molecules penetrate rapidly through the amorphous regions of macromolecules during swelling, and interact with their free binding sites or interrupt the existing intermolecular bonds. Some parts of the macromolecular chains can be fully hydrated easily and, due to thermal motion, recede from each other. This reveals additional binding sites in the chain that are also solvated and solubilised. Swelling is limited only to this stage, as the hydrocolloid absorbs only a certain amount of liquid (usually through capillary swelling); gelatine or agar particles retain their original shape and do not dissolve further. Properties of the swollen material depend on the degree of hydration and the extent of the remaining intermolecular bonds. For example, all soluble polysaccharides go through this transition state during dissolution. Agar is not further hydrated, but other polysaccharides such as gum arabic continue to swell. During further hydration, a layer of water molecules surrounds the molecule of a polysaccharide and the polymer is dispersed in an aqueous environment creating a monodispersed system. With their change in shape, the solid polysaccharide molecules dissolve and form a solution (sol).

Attractive or repulsive forces between particles generally determine the stability of sols. If the particles remain dispersed, their mutual repulsive forces are larger than the attractive forces. The size of these forces is determined by various factors. Important factors in all hydrophobic and hydrophilic sols include the chemical structure of the substances, their particle size, distance between particles, presence of electric charge (particles with the same charge are repelled, while particles with the opposite charge are attracted), other mutual non-bonding interactions of particles (mainly dipole-type interactions of all three types of dipoles), solute concentration, temperature and so on. In hydrophilic sols, interactions of polarised or ionised functional groups with water through hydrogen bonds (hydration) are of great significance.

Water molecules of hydrophilic sols, in the vicinity of dispersed molecules of high molecular weight substances (proteins and polysaccharides), are relatively tightly associated by non-bonding interactions. They are usually in the state of thermodynamic equilibrium and are therefore relatively stable. Their viscosity and surface tension are significantly different (higher) than the viscosity and surface tension of the pure dispersion medium (water).

Coagulation of dispersed particles of sols can be attributed to different factors, such as:

- increased concentration of dispersed phase;

- temperature changes;

- mechanical agitation;

- addition of substances that remove the solvation (hydration) core of the macromolecules;

- addition of a sol whose particles have the opposite electrical charge;

- addition of electrolytes that affect the electric double layer of the particles and change the value of the ζ-potential (zeta potential in the interfacial double layer); all particles of the same substance in the same environment have the same charge sign and in a collision between two particles, electrostatic repulsive forces act against the adhesion forces and no coagulation occurs.

The lowest concentration able to cause coagulation of an electrolyte is called the **coagulation threshold** and its reciprocal is the **electrolyte efficiency in precipitation** (the higher the precipitation value, the less efficient the electrolyte is in the precipitation and vice versa). The value of the ζ-potential at which coagulation occurs is the **critical potential**.

Adding small amounts of inorganic or organic salts to hydrophobic sols causes suppression of the electrical charge on the surface of the colloidal particles or in the vicinity of the particles, which leads to a decrease in the ζ-potential and coagulation. Hydrophilic sols do not coagulate with the addition of small amounts of electrolytes, but their molecules are stabilised, which is manifested by the so-called **salting-in effect**. Precipitation occurs only if the electrolyte is present in excess. The electrolyte then acts as a dehydrating agent, which is manifested by the **salting-out effect**.

The salting-out abilities of ions are different. Multivalent ions are predominantly effective (when they have opposite electric charge signs to the colloidal particles). The coagulation threshold ratios of monovalent, bivalent and trivalent counter-ions are 1 : 0.016 : 0.0015. In accordance with the declining influence of the salting-out ability, the ions of inorganic and organic salts (ions of some dyes, alkaloids, saponins and some neutral organic molecules such as sugars, which eliminate the hydration core of molecules, are effective) belong to the so-called **lyotropic series**, for example:

- anions: citrates^{3-} > tartrates^{3-} > PO_4^{3-} > SO_4^{2-} > CO_3^{2-}> acetates > Cl^- > Br^- > NO_3^- > I^-
- cations: Mg^{2+} > Ca^{2+} > Na^+ > K^+ > NH_4^+
- sugars: saccharose > glucose > xylose
- sugar alcohols (alditols) and other polyols: glucitol > glycerol.

The electric charges of some hydrophilic sols (proteins or colloidal solutions of acid polysaccharides containing carboxyl and sulfate functional groups, such as pectin or agar, respectively) are significantly influenced by the pH value. At the isoelectric point, where the net charge of the molecule is zero, the stability of the colloidal solution is low. The stability of the sol can increase or decrease with a change of pH value as the dissociated functional groups bearing charges of the same sign repel each other and simultaneously interact with additional water molecules.

Electrostatic interactions between dispersed molecules are significantly affected by the dielectric constant of the medium. A decrease in the dielectric constant caused, for example, by the addition of some organic solvents (alcohols or ketones), makes the mutual electrostatic attractive forces stronger and the dispersed particles can form clusters or coagulate. This phenomenon is employed for the isolation of some polysaccharides (such as agar, carrageenan and pectin substances) from aqueous extracts. The stability of hydrophilic sols is also affected by the concentration of the colloidal particles (they have a greater tendency towards coagulation at higher concentration) and temperature. The stability of sols can increase or decrease with increasing temperature and depends on the type of colloid.

The phenomenon known as **coacervation** frequently occurs during coagulation of lyophilic sols. The sol is stratified into two distinct liquid layers that consist of microscopic and macroscopic drops or of a continuous liquid phase.

A concentrated dispersion containing more than about 10% of solids in a liquid dispersion medium is a **paste**. Pastes have the characteristic properties of both sols and gels. For example, dough is a paste, and pastes are similarly formed by some polysaccharides (such as starch).

7.8.3.2 Gels

Gels found in foods are semi-solid materials with varying degrees of elasticity, brittleness and stiffness (rigidity). They represent two-phase dispersed systems consisting of a dispersion medium, which is a solid phase (gelling agent), usually a protein or polysaccharide. The dispersion medium is water located in the spatial (three-dimensional) gel network and associated mainly by hydrogen bonds and weak physical bonds (capillary forces). Gel formation is usually accompanied by small changes in volume, Brownian motion ceases and a certain amount of heat is released (with the exception of thixotropic gels). The mechanical properties of gels (viscosity, elasticity and plasticity) are similar to those of solids.

7.8.3.2.1 Formation

Gels are formed from sufficiently concentrated colloidal sols (solutions), usually due to changes in temperature or by the addition of salts. Most gels are formed by the cooling of sols. In this way, for example, gels of gelatine, agar and other polysaccharides are formed. In exceptional cases, the gels are formed by heating of a sol (e.g. the gel of methyl cellulose).

Protein gels

Gels are formed when partially unfolded proteins form uncoiled polypeptide chains that interact at specific points to form a three-dimensional cross-linked network. For example, a gelatine gel forms by cooling a gelatine sol (even at a concentration of about 1%), when the protein globules uncoil into long fibrous molecules (Figure 7.24), which are mutually connected via non-bonding interactions. This creates an organised three-dimensional cross-linked structure (containing 5–6 crystalline domains per one polypeptide molecule) that binds a number of water molecules. The dispersion medium, unlike in a sol, now becomes the solid phase and water becomes the dispersion medium. The sol thus creates a gel. In the formation of gels, ionic bonds mainly act between amino groups of one polypeptide chain with carboxyl groups of another polypeptide chain. Secondary bonds are hydrogen bonds between the amide hydrogen atoms and the carbonyl groups of peptide

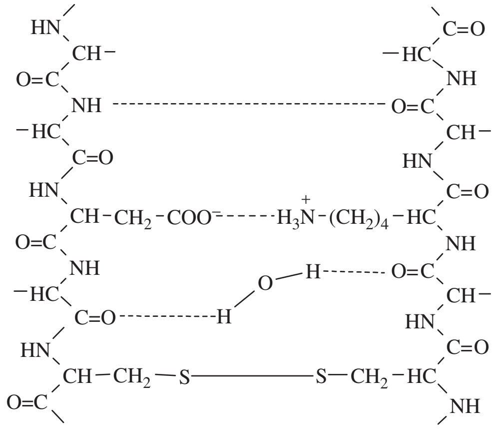


Figure 7.25 Bond types in gelatine gels.

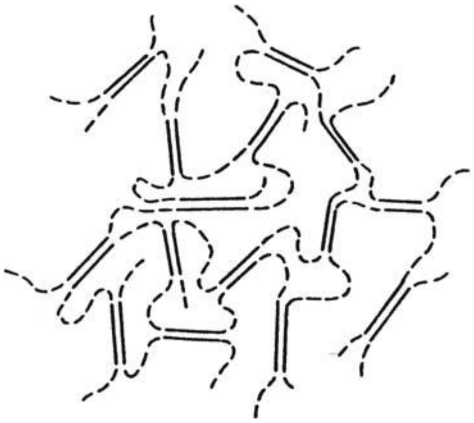


Figure 7.26 Polysaccharide gel with binding zones and randomly arranged chain residues.

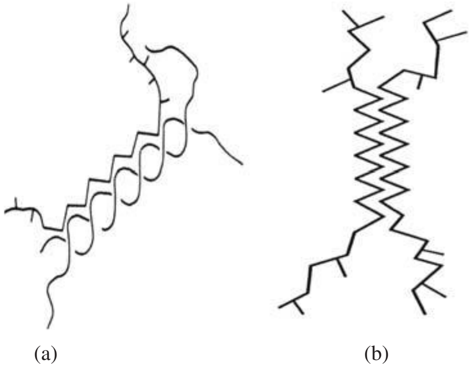


Figure 7.27 Mutual association of locust gum (a) galactomannan chains and association of locust gum galactomannan with xanthan gum (b) glucomannan chains. Stephen, 1995, fig 11.10. Reproduced by permission of Taylor & Francis – Marcel Dekker.

bonds. Polypeptide chains can also be bound by covalent disulfide bonds (Figure 7.25).

Some globular proteins denature on heating, and the denatured molecules aggregate to form gels. In addition to intermolecular ionic and hydrophobic interactions, covalent disulfide bonds also play a part in the formation of these gels. The aggregation of molecules into gels occurs at higher protein concentrations (5–10%) in the solutions. An example is the thermal denaturation of egg white. The aggregation of denatured protein molecules is caused by metal ions in some cases. An example is the formation of soybean curd (tofu) in the presence of calcium ions.

Polysaccharide gels

The mechanism of gel formation in dispersions of polysaccharides is a rather more complex process. In the sol state (at temperatures above the gel melting point), the polymer chains occur (as in gelatine) in the shape of randomly coiled globules, and gel formation on cooling occurs only if an association with neighbouring macromolecules is possible. The ability to form gels depends mainly on the primary structure of the polysaccharide. In perfectly linear unbranched polysaccharides, such as amylose, interactions through hydrogen bonds occur along virtually the entire chain lengths. As a result of this association, dilute solutions of amylose precipitate, and concentrated solutions form rigid gels that are susceptible to retrogradation during further aggregation of molecules. At the other extreme are highly substituted and branched polysaccharides, such as gum arabic, which do not form gels, but only form viscous solutions. Many other polysaccharide molecules have certain parts of the chain unsubstituted or unbranched and in these so-called **binding zones** gelation is due to the formation of intermolecular junction zones between the binding zones of different chains, which leads to the formation of gels. For example, the binding zones of locust gum are the sequences of unsubstituted D-mannose units, while the pectin binding zones are smooth regions composed of D-galacturonic acid units (Figure 7.26).

Gels known as low water activity gels or sugar–acid–pectin gels, formed from highly methoxylated (esterified) pectins that contain more than 50% carboxyl groups esterified with methanol are formed by this mechanism. A prerequisite of the gel formation is the addition of sugar (saccharose), at a concentration of least 500 g/l, which acts as a dehydrating agent. Another condition is a sufficiently acidic medium maintained by adding fruit juice or acids ($pH < 3.5$). Such a gel is considered a three-dimensional network of pectin molecules with immobilised solvent (water), sugar and acid. In this environment, free carboxyl groups are not dissociated, and therefore the otherwise charged chains are not repulsed. The three-dimensional structure formation is based on chain associations stabilised by hydrogen bonding between undissociated carboxyl and secondary hydroxyl groups, and by hydrophobic interaction between methyl esters.

The association of chains and the formation of locust gum gel and the formation of a mixed gel of locust gum with xanthan gum, where the molecules form double helical structures, is shown schematically in Figure 7.27. Like locust gum galactomannan, glucomannan of xanthan gum also forms gels with glucomannan of konjak gum.

Agars and carrageenans (also furcellaran) form gels so that the molecules first associate to double helices that are the gel

building structures. Double helices are transformed by further association to more complex helical aggregates known as superhelices (Figure 7.28). Agars form solid gels at a concentration of about 1% w/w. The average molecular weight of the basic building block of disaccharide agarose is about 150 Da; therefore each polysaccharide molecule binds at least 550 water molecules. Association of basic double-helical structures to more complex helical aggregates also creates gellan gels (Figure 7.29).

Low methoxylated (esterified) pectins can form gels in the presence of divalent cations, usually calcium. The interaction with calcium ions of fragments of two D-galacturonic acid adjacent units in two parallel chains of low methoxylated pectins is shown in Figure 7.30. Molecules of pectin with a low number of methoxyl groups (high content of free carboxyl groups) repel each other, which prevent the association of molecules via hydrogen bonds and hydrophobic interactions and the formation of gels. Therefore, gels are formed only in the presence of calcium ions. The initial strong association of two polymers into a dimer is followed by weak interdimer aggregation, mainly governed by electrostatic forces. Interactions in the entire length of the binding zones of low methoxylated pectin (also of sodium alginate salt) with calcium ions produce structures idealised in the so-called egg box-model (Figure 7.31). Alginates or gellan similarly form gels in the presence of metal ions, particularly calcium. The schematic structure of the alginate gel is shown in Figure 7.32. Binding zones comprising chain segments are mutually linked units of L-guluronic acid joined by calcium ions.

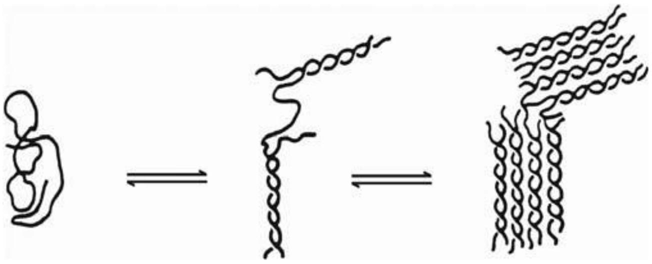


Figure 7.28 Formation of agar and carrageenan gels. Glicksman, 1982, fig 12. Reproduced by permission of Taylor & Francis – CRC Press.

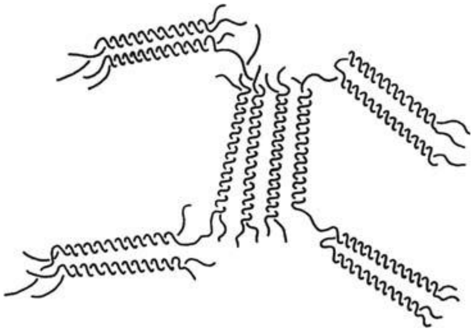


Figure 7.29 Formation of gellan gels.

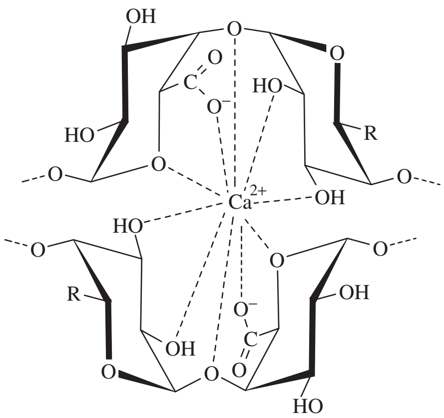


Figure 7.30 Interactions of low methoxylated pectin with calcium ions.

7.8.3.2.2 Properties

The structure of the gel is influenced by temperature, mechanical stress, pH and the presence of electrolytes (salts) and non-electrolytes (sugars). With the increase in temperature, gels become semi-solid materials, pastes or even viscous liquids. In many cases this process is reversible and gelation (e.g. of gelatine or starch gels) occurs again on cooling. Gels of this type are called

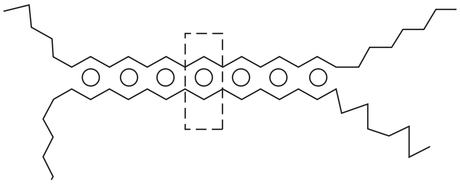


Figure 7.31 Schematic interactions of low methoxylated pectin or sodium alginate with calcium ions: O = calcium ions. Stephen, 1995, fig 10.9. Reproduced by permission of Taylor & Francis – Marcel Dekker.

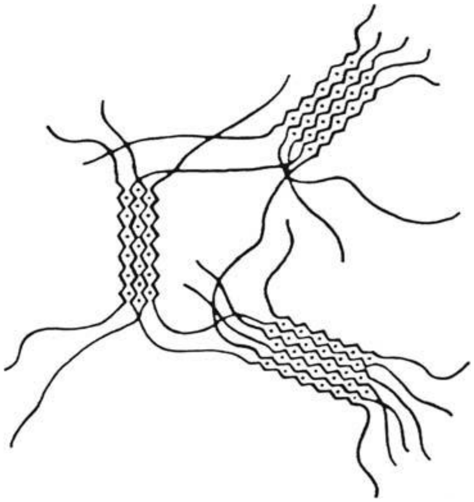


Figure 7.32 Formation of sodium alginate gels in the presence of calcium ions. Glicksman, 1982, fig 14. Reproduced by permission of Taylor & Francis – CRC Press.

thermo reversible gels. The process is related to the disappearance and appearance of hydrogen bonds between molecules of gelling agents. The opposite is **thermo irreversible gels**, in which covalent bonds (e.g. disulfide bonds) necessary for gel formation are irreversibly interrupted on heating.

The liquefaction of gels also often appears as a result of mechanical stress (when gels are shaken or agitated). If the gels that undergo liquefaction are then left to stand they rapidly regain their original consistency. This reversible property of some gels is called **thixotropy** (a time-dependent change in viscosity). These gels are called **thixotropic gels**.

Gels usually retain their original volume for a long time, but aging of some gels is manifested by **syneresis**, which is referred to as **retrogradation** if it takes place in starch gels. Aging decreases the gel volume, meaning that gel does not firmly bind all the water, which is partly released and excreted into the environment. Dried out gels are called **xerogels**. The term xerogel is also used for dried compact macromolecular gels such as gelatine gels.

7.8.3.3 Emulsions

Emulsions are heterogeneous dispersed systems (hydrophobic colloids) similar to lyophobic sols. They are the result of dispersions of immiscible liquids. One of the liquids forms a dispersed phase, which is dispersed in the liquid dispersion medium of the second liquid into small particles (drops). Depending on the size of dispersed particles and the associated stability, emulsions can be distinguished as:

- **macro emulsions** with a particle size >100 nm, typically in the range 100–1000 nm (turbid, milky and thermodynamically unstable dispersions of mutually immiscible liquids);

- **micro emulsions** with a particle size of 10–100 nm (clear and thermodynamically stable dispersions).

Emulsions in foods are macro emulsions, mainly mixtures of oil and water. The less polar phase of an emulsion (of lower relative permittivity) is referred to as the oil, while water is the second phase of the emulsion. Emulsions in food are of two types. An oil-in-water (o/w) emulsion (oil is the dispersed phase and water is the dispersion medium) contains small droplets of oil that are dispersed in water. Alternatively, a water-in-oil (w/o) emulsion has small droplets of water (dispersed phase) that are dispersed in oil (dispersion medium). Like all hydrophobic colloids, macro emulsions are unstable, because the dispersed phase tends to **coalescence** (small drops aggregate into larger drops and possibly even into a continuous phase, a layer) and the emulsions can then be divided into two phases, usually irreversibly. According to the density of the dispersion medium, the dispersed phase can be concentrated either on the surface or settle to the bottom of the container.

The stability of emulsions is influenced by many factors, such as the dispersed phase particle size (the smaller the particles, the more stable the emulsion), the amount of dispersed phase (more stable emulsions are formed with small amounts of dispersed phase), the densities of both phases (emulsions are stable when the differences between the densities of both liquids are minimal), the viscosity of the dispersion medium (emulsions with a more viscous dispersion medium are more stable), temperature (extremely high or low temperatures are undesirable), dispersed phase electric charge (stability is influenced by the presence of identical electric charges on the dispersed particles preventing coalescence), the presence of electrolytes (stability is lower in the presence of electrolytes), interfacial tension (low interfacial tension increases emulsion stability) and other factors, including mechanical stress.

Emulsions, as well as other hydrophobic colloids (such as foams), can be stabilised mechanically or by the addition of natural or synthetic surfactants called emulsifiers, which reduce surface tension at the interface of both phases. For example, the surface tension at the interface of oil–water is 20–25 mN/m. In the presence of monoacylglycerol (0.3%), it is reduced to about 80%, and by adding a mixture of lecithin and monoacylglycerol it drops to about 5% of the initial value or less. In the interface, lipophilic groups of the emulsifier molecule interact with the lipophilic (non-polar) molecules of oil and hydrophilic (polar) groups of the emulsifier interact with water. The emulsifier, for example phospholipid or monoacylglycerol, forms a protective barrier that prevents coalescence of oil drops, connects the two liquids (oil and water) and stabilises the emulsion (Figure 7.33).

The stability of emulsions, as well as of other lyophobic colloids in the presence of emulsifiers, is limited. Therefore emulsion stabilisers are often added. These can be low molecular weight hydrophilic substances (such as glycerol) or hydrophilic colloids (proteins or polysaccharides, for example, gelatine, pectin, vegetable gums or modified celluloses, respectively), which either increase the viscosity of the emulsions or interact with particles of hydrophobic colloids and thus allow their association with water.

Examples of oil-in-water emulsions are milk and mayonnaise (where the dispersion medium is water or vinegar, and the dispersed phase is milk fat or vegetable oil, respectively). The emulsifier in milk is casein, but the emulsifiers of mayonnaise are egg yolk phospholipids. An example of a water-in-oil emulsion is butter or margarine (where the dispersion medium is oil and the dispersed phase is water). Emulsifiers of butter are mainly natural phospholipids, while synthetic emulsifiers (monoacylglycerols and their derivatives and other compounds) are used to stabilise margarine.

In cases where the formation of emulsions is an undesirable phenomenon, their destruction can be achieved mechanically or chemically, for example by the addition of electrolytes.

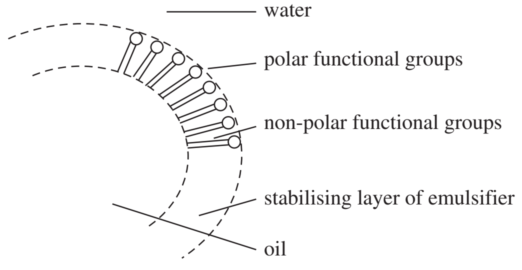
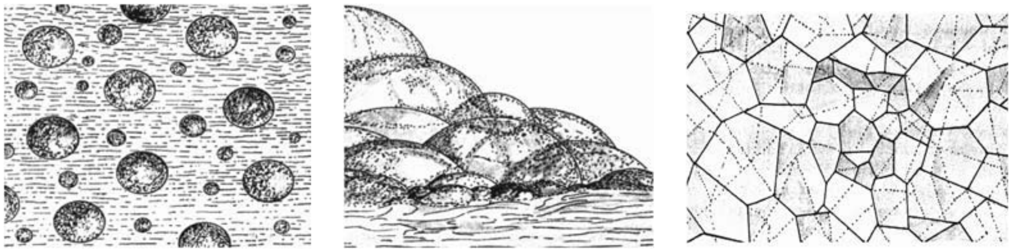


Figure 7.33 Schematic representation of an oil drop in an oil-in-water emulsion.



(a) (b) (c)

Figure 7.34 Round gas bubbles in a diluted liquid phase of foam (a), hemisphere bubbles on the liquid phase surface of a concentrated foam (b) and viewed from below (c). Zapsalis and Beck, 1985, fig 8.13. Reproduced by permission of John Wiley and Sons.

7.8.3.4 Foams

Foam is a heterogeneous dispersed system strictly similar to an emulsion, but with **foaming** as its characteristic property. Foams consist of a gas (dispersed phase) dispersed into small bubbles in the liquid dispersion medium. The individual gas particles are separated by a liquid environment of different thicknesses (a few millimetres to a film thinner than 1 nm). Bubbles are of various shapes, according to the amount of gas located in a dispersion medium and where they occur (in the liquid phase or on the surface), but are typically round or hemispheres (Figure 7.34).

Foams exhibit characteristic elasticity[15] and stiffness. Foam texture is related to the bubble size. A fine textured foam consists of small bubbles, whereas a coarse foam contains larger bubbles. As heterogeneous dispersed systems, foams are thermodynamically unstable. When they collapse, which occurs in systems consisting of only the dispersion medium and dispersed phase (e.g. in air–water or water–carbon dioxide in soda water systems), the liquid phase flows from the liquid film that forms the bubble wall within a few seconds.

The stability of foams is higher in the presence of surfactants, **foaming agents**, which adsorb onto the bubble walls and are arranged in the interphase so that their hydrophilic groups are situated in the water and their hydrophobic groups are directed into the air. They reduce the permeability of bubbles and increase the viscosity of the liquid phase, thus reducing the loss of liquid phase, which flows from the liquid film. In addition to the properties of the dispersed phase, dispersion medium and the presence of surfactants, properties of foams are influenced by other factors, such as the method of foam preparation, the gravitational force, the forces associated with collisions between bubbles, evaporation of the liquid phase and many others.

The dispersed phase of foams in foods is usually air or carbon dioxide. The gas is generally mechanically dispersed in a liquid, for example by whipping or bubbling. Foam texture affects dough and almost all bakery products, creams and whipped creams. Foams form on the surface of non-alcoholic and alcoholic beverages. For example, the properties of beer foam are closely related to the beer constituents such as alcohol, carbohydrates, melanoidins, metal ions, iso-α-acids and proteins. The foam is composed mainly of dispersed bubbles of carbon dioxide in beer (or nitrogen in the case of Velvet beer produced, for example, by Pivovary Staropramen, Czech Republic). A pivotal role in stabilising beer foam is attributed to barley proteins or polypeptides interacting with iso-α-bitter acids derived from hops that are the major contributor to the bitter taste of beer. The bubbling properties of carbon dioxide dissolved in sparkling wines consist of the bubble nucleation on tiny particles stuck on the glass wall, bubble ascent and growth through the liquid and bursting of bubbles which constitutes this most functional and visually appealing step. A foam where the dispersed phase is air is similarly produced when egg white is whipped. The stability of a foam is higher when the protein is partially mechanically denatured as in whipped egg white. Hydrophobic groups of ovalbumin are then directed to the non-polar gaseous medium and hydrophilic functional groups interact with water. Thus oriented partially denatured albumin molecules aggregate and increase the viscosity of the foam. By heating, the whipped egg white proteins coagulate producing a **solid foam**, which are found in some candies. Another example of a solid foam is ice cream, which also contains a considerable amount of air.

The naturally occurring foaming agents in foods are commonly heteroglycosides, known as saponins (that form stable foam at very low concentrations, around 0.005%), some polysaccharides and proteins. Food additives used as foaming agents include polysaccharides (e.g. plant gums) and proteins with concentrations of about 0.5–2% by weight of liquid. Many other compounds are used as food additives, such as glycerol, its ethers, primary alcohols, some saponins and other compounds. Beer, for example, may contain propylene glycol alginate as a foam enhancer.

Foaming is sometimes unwelcomed in syrups, fruit concentrates, soft drinks, vegetable oils, tea and coffee extracts and in many other commodities. Reduction of foaming in these cases can be achieved by adding certain substances to cause the collapse of the foam. Their effect depends on their tendency to form a monomolecular film on the surface, which destabilises the foam. Often these substances reduce the surface tension of the liquid phase to the threshold value at which the bubble walls are so thin that they burst. Commonly used additives are silicone oils in concentrations of 10–100 mg/kg and also primary fatty alcohols, fatty amides, fatty acids and their esters.

[15]The elasticity (E) is related to changes in the surface tension (γ), caused by deformation of bubble walls: $E = 2A.\mathrm{d}\gamma/\mathrm{d}A$, where A = surface area.

7.8.4 Properties of dispersed systems and foods

7.8.4.1 Mechanical properties

A branch of physics that is called **rheology** studies the behaviour of solids and liquids under mechanical stress, which manifests itself by deformation of solids and by flow of liquids under applied forces. The relationship between rheological and organoleptic properties of foods is the subject of **psychorheology**.

From the viewpoint of rheology, foods are very complex materials often composed of solid and liquid components. They are mixtures of various dispersion systems (homogeneous, heterogeneous, molecular, colloidal and coarse dispersions) and are not clear-cut dispersed systems. An example of such a complex material is milk (see Section 2.4.5.2). The behaviour of foods under mechanical stress (rheological behaviour) is closely related to their **texture**. The term texture includes properties of food producing the tactile (haptic) perception of tactile receptors, registered in the oral cavity and often applied by the touch of hands. The consumer assesses, for example, the hardness of fruit according to the deformation resulting from a finger pressure or force that must be employed on chewing. The physical state and physico-chemical properties of foods are very important attributes, as they affect food behaviour during processing, storage and consumption.

The term **consistency** is used to describe the mechanical aspects of the texture associated with the physical properties of food. Important rheological properties of solids are **elasticity** and **plasticity**, while an important feature of fluids is **viscosity**. These phenomena are responsible for the characteristic texture and properties of many foods, since foods often simultaneously exhibit the properties of elastic solids and viscous liquids. Elasticity is the property of a material that enables it to resume its original shape or size when the mechanical stress is removed. Plasticity is the ability of the material to be the subject of a continuous and permanent deformation when exposed to external, usually small forces that exceed the elastic limit of the material (the stress point at which a material subjected to higher stress will no longer return to its original shape and will deform or break). The degree of plasticity depends on various factors such as temperature. By increasing the temperature, an elastic gel, for example, is transferred from the elastic to the plastic (viscoplastic) state. The transition to the plastic state can be realised not only by heating, but also by the addition of water, which acts as a plasticiser. The plastic state of a material has certain characteristic and specific properties that are similar to those of both solids and liquids. Plasticity is to some extent related to viscosity, which is a characteristic property of liquids. Elasticity and viscosity are distinguishing properties of some dispersed systems (such as gels) and many other foods (e.g. dough and bread). Often, therefore, the mechanical properties of foods are described as **viscoelastic properties**. Geometric texture attributes, such as shape and size, are often called **appearance**. The appearance of foods is also closely related to their colour.

Various descriptive terms are used to characterise texture. For example, hardness means resistance to deformation; firmness is essentially identical to the term hardness, and is sometimes used to express the ability to resist deformation caused by the material's own weight. Cohesiveness relates to the strength of internal ties of the material. Flexibility refers to the speed with which the deformed material regains its original shape when it is not subjected to the deforming force (it is associated with a relaxation time of the restoration to the original shape). Related terms are toughness, fragility, brittleness, friability and crispness. Stickiness is the adhesion between the surface material and the adjacent surface. If both surfaces are of the same material, then the correct term is cohesion. The mechanical characteristics are also described by terms such viscosity, pastiness, thinness and other terms. Responsible for foods properties reported by these terms are generally macromolecular food components such as proteins, polysaccharides and products of their mutual interactions and their interactions with other food constituents, first and foremost with water. The water content (amount of dry matter) and fat content in a food are related to the terms dry, watery, greasy and tallow, and to many other synonyms.

7.8.4.1.1 Elasticity

An important property of many materials is their ability to regain their original shape after the load is removed. These materials are called elastic. When an external force acts on a material (external forces are usually much greater than the forces caused by its own weight), it can cause a change in the state of its motion (e.g. change of location or rotation) or deformation, the scope and duration of which depend on a number of factors. When the deforming force is weak (between zero and the elastic limit) and acts for a short time, it generally leads to elastic deformation, which is temporary and the material will regain its initial dimensions. When the deforming force is weak, but acts for a long time or exceeds the elastic limit of the material, the typical result is non-elastic (permanent) deformation. The ratio between elastic and non-elastic deformation is called the degree of elasticity.

The deformation of an ideal solid material under a load is expressed by Hook's law. Deformation can change the material dimensions. For example, the extension (strain) (ε) of a string of the original length (l) caused by stress applied is (Δl). The relative extension of the string $\varepsilon = l/\Delta l$ is in direct proportion to its tensile stress (σ) applied (which is uniaxial) and in indirect proportion to the Young's modulus (E), also known as the modulus of elasticity ($\varepsilon = \sigma/E$, E = stress/strain = σ/ε), which characterises the rigidity of the material. Deformation may be caused not only by pulling or compression, but also by more complex phenomena such as bending and torsion.

A higher value of the modulus of elasticity indicates a rigid material (the E value is about 200 000 MPa for steel), a lower value indicates a more brittle material (such as glass, where E = 50 000–90 000 MPa), and a very low value represents a ductile material (such as rubber with E = 10–100 MPa). The E values of certain foods are, as an illustration, listed in Table 7.9. These values depend on many factors. For example, the modulus of elasticity in bread changes during storage and depends on the type of flour and addition of emulsifiers and a range of other variables.

A similar relationship as for the uniaxial stress caused by pressure is also true for the shear stress (τ) (tension force components

Table 7.9 Modulus of elasticity values of some foods.

Food	Modulus of elasticity (MPa)	Food	Modulus of elasticity (MPa)
Bread (fresh)	0.005	Bananas	0.8–3
Bread (almost fresh)	0.01	Apples, potatoes	6–14
Bread (old)	0.02	Carrots	20–40

act in the tangential direction) and deformation γ of the solid homogeneous isotropic material by the shear stress: $G = \tau/\gamma$, where G = shear modulus or modulus of rigidity is the ratio of shear stress to shear strain. The elastic moduli E and G (measures of stiffness) are correlated by the equation $E = 2G(1 + \nu)$, where ν (Poisson's ratio) is the ratio of relative transverse shortening (contraction) and relative longitudinal extension strains of the material in the direction of stretching force expressing the phenomenon that the material stretched in one direction tends to get thinner in the other two directions.

7.8.4.1.2 Viscosity

The ideal fluid differs from the ideal elastic solid as the shear stress causes irreversible deformation manifested by fluid motion (flow). The liquid is trying to hinder the relative movement of one layer of liquid molecules relative to the other layers. It therefore has some internal friction (internal resistance), which is related to the mutual interaction of liquid molecules. This friction is called shear **viscosity**, often referred to simply as viscosity, which is a measure of a fluid's resistance to flow.

An ideal fluid has constant viscosity when the value of the operating shear stresses changes. When the operating shear stress (σ) is uniaxial, the relationship is analogous to that of solids:

$\lambda = \sigma/\dot{\varepsilon}$, where λ = extensional (elongational) viscosity, a viscosity coefficient when applied stress is extensional stress (σ), $\dot{\varepsilon} = d\varepsilon/dt$ = change of deformation in time or strain rate. This parameter is often used for characterising polymer solutions.

Dynamic (**absolute**) **viscosity**, or the coefficient of absolute viscosity (η), is the measure of internal friction of the liquid (viscosity) at shear stress (τ) between the layers of non-turbulent fluid (Newtonian fluid) moving in straight parallel lines, which is given by the equation:

$$\eta = \tau/\dot{\gamma}$$

where $\dot{\gamma} = d\gamma/dt$ = shear strain rate. Dynamic viscosity of a Newtonian fluid (η) is a constant in the equation (Newton's law of viscosity or Newton's law of friction and motion): $\tau = F/A = \eta.dv/dx$, where τ = shear stress in the direction of the x-axis, caused by the force (F) between two planes (layers) of the liquid, A = area of planes, dv/dx = shear stress or rate gradient between the planes perpendicular to both planes. Specific equations have also been proposed for non-Newtonian fluids.

The reciprocal of the dynamic viscosity, the fraction $1/\eta$, is called **fluidity**. The proportion of dynamic viscosity and fluid density (ρ) is called **kinematic viscosity** (ν): $\nu = \eta/\rho$.

The viscosity of a fluid depends on pressure and temperature. With increasing pressure, viscosity increases, but the effect of pressure (with the exception of high pressures) on the viscosity of a fluid is usually negligible. With increasing temperature the viscosity of a fluid generally decreases substantially. Several equations expressing the dependence of viscosity on temperature have been suggested. Probably the most successful equation is: $\ln\eta = A + B/T$, where A and B = constants and T = absolute temperature.

Most low-molecular weight fluids obey Newton's equation and therefore are called **Newtonian fluids**. Their viscosity is constant at a given concentration of a substance in solution, and at the given temperature does not depend on the shear rate (dv/dx). The only parameter is viscosity, which characterises these liquids completely rheologically. The relationship between viscosity and concentration of dispersed phase for Newtonian fluids can be expressed by a number of equations.[16] The viscosity of fluids decreases exponentially with increasing temperature due to the decrease of interactions of molecules and their greater distance.[17] The dependence of the viscosity of aqueous solutions of saccharose on concentration and temperature is illustrated in Figure 7.35.

The viscosity of polymer dispersions depends, amongst other factors, on the structure of the macromolecules. Dispersions of soluble linear polysaccharides (such as amylose) are more viscous than dispersions of substituted polysaccharides (such as guar gum) and the less viscous dispersions are formed by branched polysaccharides (e.g. amylopectin and gum arabic). Viscosity is generally a function of radius of gyration,[18] Radius of gyration is related to the volume

[16]The viscosity of dispersed systems is different from the dispersion medium viscosity (pure liquid). To establish these deviations in Newtonian liquids, the following relations are used: the relative viscosity $\eta_r = \eta/\eta_0$ (η = viscosity of dispersed system, η_0 = viscosity of pure dispersion medium), the specific viscosity $\eta_{sp} = (\eta - \eta_0)/\eta_0 = 1 - \eta_r$ or viscosity number η_{sp}/c (c = dispersed phase concentration) and limiting viscosity number $[\eta] = \lim_{c\to 0} \eta_{sp}/c$. In dispersed systems, where the dispersed phase particle sizes are comparable to the molecular dimensions of the dispersion medium, the following equation can be used: $\ln\eta = x_1.\ln\eta_1 + x_2.\ln\eta_2$, where η_1 and η_2 = viscosities of components, x_1 and x_2 = their mole fractions.

For the viscosity of diluted dispersed systems with spherical particles, large in comparison with the dispersion medium particles, Einstein's equation was derived: $\eta = \eta_0.(1 + K.\varphi)$, where the constant $K = 2.5$ and φ = dispersed phase volume fraction. Deviations from Einstein's equation (where $K > 2.5$ for non-spherical particles or solvated lyophilic sols) expresses the so-called rheological voluminosity: $k.V_0 = \lim_{\varphi\to 0} \eta_{sp}/2.5{*}\varphi$. For more concentrated dispersed systems other equations are used. The relationship between the relative molecular weight (M_r) and limiting viscosity number is given by the Mark–Houwink equation: $[\eta] = K.M_r^{\,a}$, where K and a = empirical constants. This equation allows the determination of the relative molecular weight of high molecular weight substances, such as proteins and polysaccharides.

[17]The dependence of viscosity on temperature for $T > T_m$ (melting temperature) is the Arrhenius equation: $\eta = A.\exp(E/RT)$, where A = pre-exponential factor (pre-factor), E = activation energy of flow, R = universal gas constant, T = absolute temperature (this relationship does not apply at temperatures $T < T_m$).

[18]The radius of gyration $\sqrt{s^2}$ is the root mean square distance of the individual parts of a macromolecule from its centre of gravity. The relationship between the radius of gyration and the root mean square distance in random balls of linear chains is given by a simple equation: $\sqrt{s^2} = \sqrt{r^2}/\sqrt{6}$.

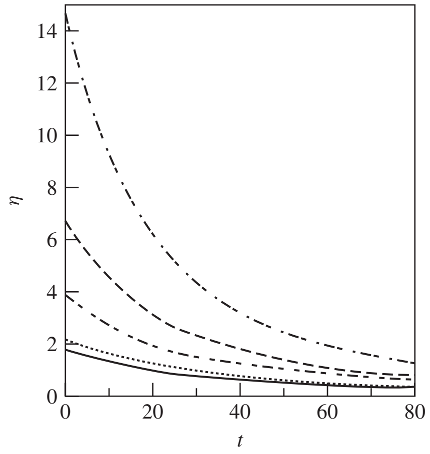


Figure 7.35 Dependence of dynamic viscosity of aqueous saccharose solutions on the concentration and the temperature: η = dynamic viscosity (mPa/s), t = temperature (°C), —— = 0% (water), ⋯⋯ = 10%, – – – = 20%, – – – –· = 30%, —· · —· · = 40% saccharose solution.

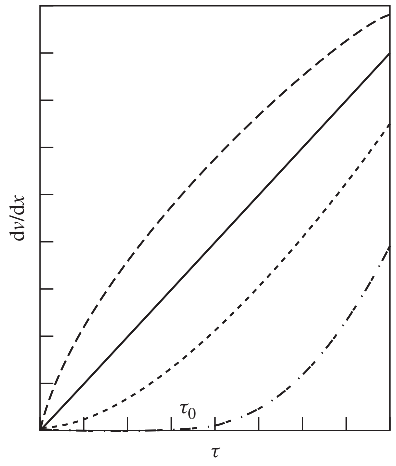


Figure 7.36 Schematic representation of shear rate (dv/dx) of Newtonian and non-Newtonian fluids on shear stress (τ): —— = Newtonian, – – – – – – = dilatant (shear thickening), ⋯⋯ = pseudoplastic (shear thinning), —·—·—· = plastic fluid, τ_0 = yield stress. Zapsalis and Beck, 1985, fig 8.24. Reproduced by permission of John Wiley and Sons.

that the molecules occupy in a dispersion medium under thermal motion. Linear branched molecules of about the same number of monomers have a much larger radius of gyration (Figure 7.12).

Newton's equation is not valid for **non-Newtonian** (non-ideal or real) **fluids** that contain asymmetrical particles, clusters of molecules or macromolecules. Many common food systems, such as suspensions, emulsions and gels, belong to non-Newtonian fluids. Their viscosity depends on the shear rate (dv/dx) or the tangential force. However, Newton's law also applies to certain non-Newtonian fluids at smaller flow rates and to dilute polymer solutions (such as hydrophilic sols) at very low shear stresses. The rheological characterisation of non-Newtonian fluids requires knowledge of the dependence of shear stress on shear rate, which is called the **flow curve**. For example, the real shear strain rate ($\dot{\gamma}$) for spreading butter, margarine or processed cheese on bread is 0–100 s^{-1}, when pouring liquids from bottles 50–200 s^{-1}, when pumping liquid chocolate in a pipe of a diameter of 100 mm and at a flow rate of 50 l/min is 30 s^{-1}.

The non-Newtonian fluids are classified according to behaviour that they show depending on the rate gradient (dv/dx) and the tangential force (Figure 7.36). The following five non-Newtonian fluids (systems) are recognised:

- **plastic** (Bingham plastic)
- **pseudoplastic** (shear thinning)
- **dilatant** (shear thickening)
- **thixotropic**
- **rheopectic**.

Plastic systems initially behave as solid (Bingham) bodies (for $\tau < \tau_0$) and resist deformation until a yield stress (τ_0) is reached. When that stress is exceeded, the shear rate grows. Further stress leads finally to linear (Newtonian) behaviour. Examples of plastic systems are chocolate, butter, cheese, various spreads and ice cream. In pseudoplastic systems the observed viscosity decreases with an increase in shear stress. An example of a pseudoplastic system is pudding. Dilatant systems resist deformation more than in proportion to the applied force. The shear rate is growing much faster than that of Newtonian fluids and viscosity increases with an increase in shear stress. At low applied forces, the system behaves as a Newtonian fluid. Examples of dilatants systems are honey with added dextran and a slurry of wet beach sand. Thixotropic systems become more fluid (they have lower viscosity) with increasing time of an applied force. If the applied force ceases to operate, the original viscosity of the system is restored due to a reversible transformation of the sol–gel type. Examples of thixotropic systems are mayonnaise, ketchup, whipped and hardened fats, butter and processed cheeses. Rheopectic systems exhibit behaviour opposite to that of thixotropic systems. Their viscosity increases with increasing time of applied force. An example is whipped egg white.

7.8.4.1.3 Viscoelasticity

The majority of foods show viscoelastic properties. This means that these foods react, when exposed to applied force, by both the elastic component (which behave as in solids) and the viscous component (which behaves as in liquids). The rheological characterisation of these materials, in addition to flow curves, requires knowledge of other parameters, complex (dynamic) modulus G^*, which is related to the elastic component of the material (storage modulus G') and the viscous component of the material (loss modulus G'') by the following equation: $G^* = (G'^2 + G''^2)^{1/2}$. With the so-called complex dynamic viscosity (η^*), which is analogous to the dynamic viscosity resulting from Newton's law, the complex modulus (G^*) is

related by the equation: $\eta^* = G^*/\omega$, where ω = oscillation frequency or strain rate under dynamic (sinusoidal) material loading.

An example of the mechanical spectra (dependences of G', G'' and η^* on ω; the SI physical unit is the Pascal-second, Pa.s, equivalent to N.s/m^2 or kg/m s) of a polysaccharide gel is shown in Figure 7.37. The gel has a much higher value of the storage modulus G than the value of the loss modulus G$''$ and the gel therefore essentially behaves as a solid. Both modules are independent of strain rate (ω) and the gel is therefore highly elastic. The complex dynamic viscosity (η^*) decreases with increasing strain rate (ω) values as the gel becomes more fluid, which means that it is thixotropic.

Important processes taking place during the formation of a gel, and the influence of temperature and concentration of the gelling agent, are shown in Figure 7.38. After an initial delay during which the system behaves as a liquid, at time t_g the gel formation starts, which is rapid at first and then slower till a certain constant value of the storage modulus G' is achieved, which is a measure of elasticity of the material. The dependence of the storage modulus G' on the polysaccharide concentration in solution (for $t \gg t_g$) shows that the gel formation occurs only at concentrations that are higher than the critical concentration c_0. At concentrations higher than c_0, the gel rigidity grows rapidly, at lower concentrations than c_0 the polysaccharide does not form a gel, and its solution remains just as a viscous liquid.

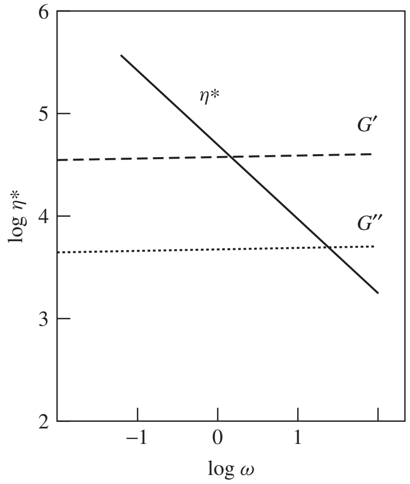


Figure 7.37 Typical mechanical spectra of polysaccharide gels: η^* = complex dynamic viscosity (Pa.s), G' = storage modulus (Pa.s), G'' = loss modulus (Pa.s), ω = oscillation frequency (rad/s). Stephen, 1995, fig 16.1. Reproduced by permission of Taylor & Francis – Marcel Dekker.

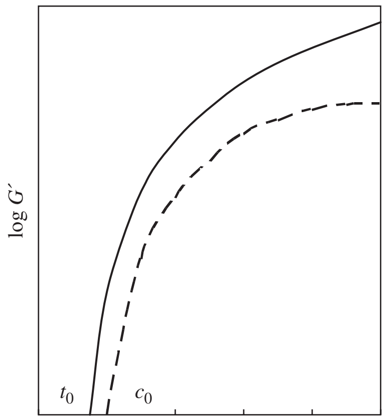


Figure 7.38 Influence of temperature and concentration on polysaccharide gels formation: G' = storage modulus (Pa.s), —— = temperature, – – – – – – = concentration. Stephen, 1995, fig 16.2. Reproduced by permission of Taylor & Francis – Marcel Dekker.

7.8.4.2 Kinetic properties

Kinetic properties of dispersed systems (dispersions) determine the rate of many processes that affect many aspects of food quality.

The dispersed phase particles of the colloidal and coarse dispersions and also of foods are in a permanent random motion called **Brownian motion**.[19] The movement of molecules is caused by bumps of the dispersion medium molecules (mostly by water molecules) that perform the thermal motion. The speed of the particle movement increases with increased temperature and decreases with particle size. At a certain particle size (approximately >4000 nm), their movement is not observable. The thermal motion of dispersed particles is the essence of some phenomena occurring in colloidal dispersion and coarse dispersed systems, and thus also in foods. These phenomena primarily include **diffusivity**, **sedimentation** due to gravitational or centrifugal forces and **osmosis**.

7.8.4.2.1 Diffusivity

In dispersed systems with a dispersed phase or dispersion medium concentration gradient (chemical potential gradient), a spontaneous process occurs to balance the concentration. This kinetic behaviour, the ability of a substance to undergo diffusion, is called **diffusion**. The speed of diffusion is characterised by the diffusion coefficient (D). Its value increases with increasing temperature and decreases with increasing viscosity of the dispersion medium and radius of dispersed particles.[20] The diffusion rate of the dispersed phase is therefore highest in molecular dispersed

[19] Mean translational energy of dispersed particles (ε_{kin}) is given by the equation: $\varepsilon_{kin} = 1/2m.u^2 = 3/2k_B.T$, where m = particle mass, u^2 = mean square speed of movement, k_B = Boltzmann constant and T = absolute temperature. The movement of particles in a certain direction (e.g. along the x-axis) is characterised by the mean shift (Δ): $\Delta=[\Sigma\Delta_i^2/n]^{1/2}$, where Δ_i = projections of individual particle displacements on the given axis and n = number of projections.

[20] One-dimensional diffusion in a stationary liquid medium is described by the first Fick's law. It shows that the diffusion flux of a substance (J) or amount of substance in moles (dS) per area (A) within a certain time (dt) is proportional to the concentration gradient (dc/dx, where dc = change in concentration of a substance at a distance x): J = dS/dt = –$D.A.$dc/dx. The non-stationary diffusion (the concentration gradient changes with time) is described by the second Fick's law as follows: dc/dt = D.$d^2.c$/dt^2. Diffusion coefficient D (in m^2/s) is related to the medium shift (Δ): $\Delta = (2D.\Delta t)^{1/2}$, where Δt = time at which the dispersed particles diffuses to distance Δ. Diffusion coefficient D is related to the friction coefficient f, which counteracts the motion of particles in the dispersion medium: $D = k_B.T/f$ (Einstein relation), where k_B = Boltzmann constant and T = absolute temperature. If the dispersed phase particles are spherical particles

systems, lower in colloidal dispersion systems and immeasurable in coarse dispersions.

The most important process in foods is diffusion of water molecules, which migrate between the non-homogeneous areas of food during storage and processing. Diffusivity determines the rate of drying, the rate of crystallisation of ice during freezing and many other processes. In foods with low moisture content, diffusion of water depends mainly on the water content. The lower the water content, the lower the diffusion coefficient. At the critical water content, which corresponds roughly to the monomolecular layer surrounding the polymers, the diffusion coefficient is zero. In partially crystalline materials such as polysaccharides (starch granules or cellulose), diffusion occurs only in amorphous regions. Another important process is the diffusion of water through food packaging.

7.8.4.2.2 Sedimentation

Sedimentation is the process by which the dispersed phase particles (large molecules or macroscopic particles) are concentrated under the action of gravitational or centrifugal forces in the direction of the force. Friction acts against this movement and diffusion can also be applied. The sedimentation rate is generally inversely proportional to the viscosity of the medium, and directly proportional to the density difference between the dispersed phase and the dispersion medium, and to the square of particle diameter.[21]

Diffusion of particles is immeasurable in coarse dispersions, where only the forces of gravity and friction act. Large particles settle faster than small particles. The movement of particles in the steady state is uniform. Molecular dispersions, on the other hand, do not show any measurable sedimentation, as the sedimentation rate is low, therefore the molecular dispersions are kinetically stable and the rate of diffusion is large. In colloidal dispersions, both processes can be balanced and may establish sedimentation equilibrium in which the sedimentation rate is equal to the diffusion rate in the opposite direction. Such a dispersion system is then kinetically stable.

In practice, the sedimentation of food particles in coarse dispersions (such as some dressings) may be prevented by the increase of the dispersion medium viscosity using suitable polysaccharides, such as carrageenan.

of comparable size with dispersion medium particles, then: $D = k.T/8\pi.\eta.r^3$, where η = dynamic viscosity of the dispersion medium and r = hydrodynamic radius of dispersed particles. Diffusion of large dispersed particles, such as biopolymers, in homogeneous diluted or concentrated solutions is described by the Stokes–Einstein relation for rotational diffusion coefficients: $D = k.T/8\pi.\eta.r^3$ and for translational diffusion coefficients: $D = k.T/6\pi.\eta.r$. From the known value of (D), it is possible to calculate the size of dispersed particles (r) or (after adjusting) their molar weight (M, in kg/mol, which is proportional to the relative molecular weight $M = 0.001.M_r$): $M = 4\pi.N_A/3v.(k.T/6\pi.\eta.D)^3$, where N_A = Avogadro's number (constant) and v = partial specific volume of particles ($1/\rho$). Other relationships are used in gaseous dispersion media.

[21]The sedimentation rate (u) is given by the following relations: $u = V.g/f(\rho-\rho_0) = m.g/f\ \ (1-\rho_0/\rho) = M.g/N_A.f\ \ (1-\rho_0/\rho) = 2r^2.g/9\eta\ \ (\rho-\rho_0)$, where V = volume of sedimentation particles, ρ = their density, ρ_0 = density of dispersion medium, f = friction coefficient, M = molar weigh of particles, N_A = Avogadro's number, η = dynamic viscosity of dispersion medium.

7.8.4.2.3 Osmosis

Osmosis is a process in which dispersion medium (solvent) particles, for example water molecules, migrate from the region of higher concentration (higher chemical potential) to a region of lower concentration (lower chemical potential) through a semi-permeable membrane. This membrane is permeable only to dispersion medium particles and is impermeable to dispersed phase particles. The solvent migration tends to increase the solvent volume of the region of lower concentration and raises the so-called **osmotic pressure** of the solution.[22] Osmotic pressure is the same as the pressure to which the solution would have to be exposed to prevent the solvent penetration through the membrane. It is directly proportional to temperature and inversely proportional to the weight of dispersed particles (their molecular weight and size). Osmotic pressure, for example, decreases if the dispersed phase particles form aggregates or when the particle is present as a polymer.

This is an extremely important phenomenon in living cells. Elastic semi-permeable cell membranes release water, but are selective to solute transport (sugars, organic acids and other substances). Very low concentrations of dissolved substances present in the cells produce high osmotic pressures that are necessary to maintain the internal pressure, called **turgor**, and a state of tension in animal tissues and plant tissues. The cells prevent too great an osmotic pressure by the synthesis of polysaccharides from monosaccharides (e.g. starch from glucose). Osmotic pressure is also an important phenomenon in animal cells post mortem and in the cells of plant tissues at the time of harvest. Higher osmotic pressures are deliberately used for growth control and inhibition of microorganisms in canned products (e.g. brines high in salt or sucrose).

7.8.4.3 Thermal properties

At higher temperatures, in frozen foods and in foods with low water content, macromolecular substances such as proteins and polysaccharides exist mainly in an amorphous state containing unorganised molecules. Amorphous or partially amorphous structures in foods commonly arise during various heat treatment processes such as cooking, concentration, drying and extrusion, where water is removed relatively quickly or concentrated dispersions are rapidly cooled. Amorphous materials are found in a non-equilibrium state and therefore, in relation to time, undergo various changes of their physical and other properties. For example, amorphous lactose crystallises in powdered milk.

When a fluid or melt (such as a sucrose melt) cools, the kinetic energy of the molecules decreases until the motion of the molecules is so small, at a certain temperature $T = T_m$ (melting temperature),

[22]In a sufficiently diluted solution, the osmotic pressure (π) is described by the following equation: $\pi = w.k_B.T/m = w.R.T/M$, where w = weight concentration of dispersed phase particles, k_B = Boltzmann constant, T = absolute temperature, m = average particle weight, R = universal gas constant, M = particle molar weight. Other relationships have been proposed for non-ideal solutions.

Osmotic pressure varies with dispersed phase particle size. Even small differences in particle size result in large differences in osmotic pressures. In two dispersions of the same concentration of particles (w), but differing in particle size, the ratio of osmotic pressures is: $\pi_1/\pi_2 = r_1^3/r_2^3$, where r_1 *and* r_2 are the radii of particles in both systems.

that the molecules can no longer escape from the secondary valence forces of neighbouring molecules, and the liquid or sucrose melt crystallises. The volume of solution or melt changes in a step (usually decreases). If the fluid or sucrose molecules become components of crystals, their spatial position is fixed, the molecules loose the freedom of movement and Brownian motion ceases.

If water, other liquids, sucrose solution or sucrose melt are cooled rapidly, it may be that they do not crystallise for kinetic reasons. Generally, cooling the fluid to a temperature below its melting point ($T < T_m$, the process is known as supercooling) changes its mechanical properties, and its viscosity increases depending on the structure of the substance. The fluid passes the region of the iso phase transition temperature (T_f) to the elastic state, which is the state of fluid with a fixed structure.[23]

Further cooling to temperatures close to T_g (glass transition temperature) results in a dramatic increase in viscosity, and at temperature $T = T_g$ the material passes from the fluid to the glassy state. The process is also called nitrification of fluids. At T_g and lower temperatures, the viscosity of vitreous material remains constant and very high (about 1×10^6 MPa/s). Other properties, such as permittivity, refractive index, diffusivity and elastic modulus are also step changes. Between the elastic state and glassy state (also known as the supercooled or solid melt) there is no difference in the structure, and only the **relaxation time** (the rate of changes of mechanical properties) characteristic for the elastic state is extended. The transition from the elastic to the glassy state is a reversible and kinetic process, since the value of T_g depends on the cooling rate.

The T_g values of food materials range from –135 °C (T_g of water) to values in the tens of °C or more above the melting point of ice (e.g. the T_g of sucrose is about 62 °C). A decrease in T_g values is seen in the presence of water and other low molecular weight compounds that act as plasticisers (e.g. the T_g value of 50% aqueous solution of sucrose is about –100 °C). The T_g value of mixtures of two or more substances lies between the T_g values of pure substances. The changes in the physical state of sucrose solutions at various concentrations and temperatures are described in the state diagram given in Figure 7.39.

Similarly to sucrose, other low molecular weight substances, synthetic polymers and polymeric compounds present in foods (proteins and polysaccharides) may also exist in various physical states, such as crystalline, glassy, elastic or plastic, among which there are iso phase transitions given by temperatures T_m, T_f and T_g. All polymers have similar properties, differing only in the

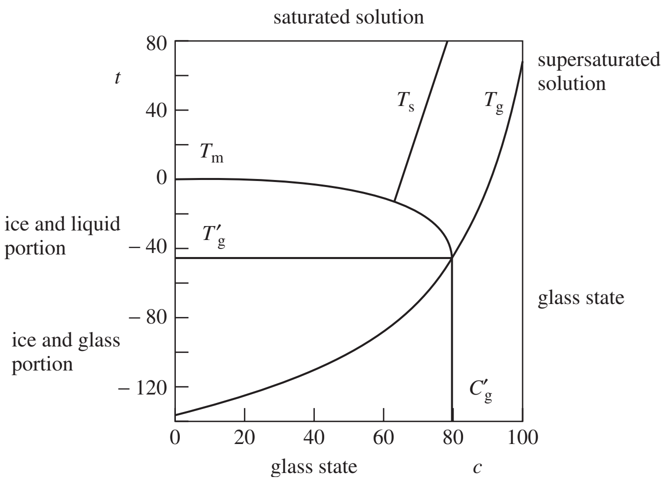


Figure 7.39 Saccharose-water state diagram: t = temperature (°C), c = concentration, T_m = equilibrium: formation-melting of ice (equilibrium melting temperature of ice), T_s = equilibrium: saturated solution-oversaturated solution, T_g = glass transition temperature, T'_g = glass transition temperature of maximum concentrated liquid phase with saccharose content C'_g

temperatures where the iso phase transitions occur. Fully crystalline solids do not have the T_g temperature and amorphous substance does not have the T_f temperature. The T_g value has a great influence on the degree of crystallinity of polymers. The value of T_g of an anhydrous material is significantly reduced in the presence of water (similarly to the synthetic polymers in the presence of plasticisers and solvents). Most biopolymers have similar T_g values. The T_g values of anhydrous substances lie in the range of 200 ± 50 °C (for starches within 151–243 °C). A decrease of the T_g value by 10 ± 5 °C is caused by the addition of 1% w/w of water. Biopolymers containing 20 ± 5% of water have the T_g value at room temperature, while biopolymers with 25–30% of water content have the T_g value at -10 ± 5 °C. Generally, the T_g value increases with increasing relative molecular weight. Up to the relative molecular weight of about 104 Da, the T_g value increases linearly with its reciprocal value.

Knowledge of the T_g values and how T_g changes depending on the water content is important for predicting the shelf life of many foods and ongoing changes related to the organoleptic properties and other quality features. Foods in the T_g region, as well as solutions or melts of sugars, suddenly change many properties, including modulus of elasticity, which increases by several orders of magnitude, and the rate of relaxation of mechanical tension. Glassy material changes to the liquid (melt) or viscoelastic material when heated to a temperature above T_g.

Viscoelastic behaviour is seen in bread, for example, which is actually a rigid foam. Typical bread containing 40% of water is glassy and brittle at temperatures below approximately –15 °C, as it is at temperature $T < T_g$. Bread can be stored at this temperature, with no retrogradation of starch or related consequences (aging and hardening) as there is under storage at normal temperatures. With increasing temperature (or water content, which acts as a

[23]At temperatures $T > T_m$ (melting temperature), the dependence of viscosity on temperature is controlled by the Arrhenius equation. In most materials, in the temperature range from T_m to T_g (glass transition temperature), the temperature decrease results in an increase of activation energy (E), which relates to the fact that molecules do not move as individuals, but in a coordinated manner. At $T > T_g$, viscosity is satisfactorily described by the so-called VTF (Vogel–Fulcher–Tammany) equation: $\eta_T = A.\exp D.T_0/(T–T_0)$ or WLF (Williams–Landel–Ferry) equation: $a_T = \exp [C_{1g}.(T–T_g)]/[C_{2g}(T–T_g)]$, where η_T = viscosity at temperature T, a_T = ratio of viscosities at T and T_g, or the ratio of relaxation times τ and τ_0 at temperatures T and T_0 and A, D, T_0, C_{1g} and C_{2g} are constants. Parameters C_{1g} and C_{2g} are considered universal constants ($C_{1g} = -17$ is for different materials virtually identical).

plasticiser), at $T = T_g$ the elasticity of bread increases rapidly and reaches a value, which remains approximately constant during further rise in temperature (or water content). Bread again becomes a viscoelastic material, but at $T > T_g$ the aging process rapidly accelerates. Some bread types (e.g. crisp bread in the Nordic countries, or German Knäckebrot) are glassy and brittle and durable even under normal temperatures, as they contain less than about 14% of water. Their glass transition temperature is higher than the ambient temperature, and therefore such breads can be stored for a long time without any changes. Wetting associated with the decrease of T_g value below room temperature occurs in extruded products, which results in the loss of crunchiness.

7.8.4.4 Optical properties

When an electromagnetic wave (an incident light ray) passes through an environment where it encounters an obstacle or an area of non-homogeneity, for example scattering particles of dispersed systems (such as in emulsions or sols), the light is scattered.[24] The intensity of light scattering in dilute dispersions on electrically non-conductive spherical particles, which is small in comparison with the wavelength of light ($<\lambda/20$), also depends on the wavelength of the incident light.[25] Light scattering in the visible spectrum region (approximately 400–700 nm) is about ten times more intense for light of shorter wavelengths (violet and blue) than for light of longer wavelengths (yellow and red). The dependence of the scattered light intensity on the wavelength is called **opalescence**. Dispersions composed of very small particles show weak opalescence, while dispersions of large particles do not show opalescence, because the light is reflected.

Opalescence often significantly affects the colour of dispersions. Dispersions exhibit different colours in incident and transmitted lights. These colours are complementary to each other; the blue part of the spectrum predominates in diffuse light and red light predominates in the transmitted light. Therefore, diluted milk is pink as it leads to scattering of light of shorter wavelengths, while light of longer wavelengths passes through the dispersion (milk).

7.8.4.5 Electric properties

Dispersed systems are externally electroneutral as a whole, but show some electric (electrokinetic) properties. Electrical properties of colloidal dispersion systems are associated with the ability to disperse particles that adsorb ions and molecules of the dispersion medium. The important food dispersed systems, lyophilic sols, preferentially adsorb ions resulting from dissociation of electrolytes. Electric charges of the same sign are then accumulated on the surface of colloidal particles as a result of adsorption of the ions that form an inner layer of ions. The electric charges (ions) of opposite sign, which constitutes the outer layer of ions, are in the surrounding part of the dispersion medium. Around the colloidal particles an **electrical double layer** is thus formed that stabilises the sols. Ions of the outer layer are arranged in two different sub-layers. The first section of ions, partially neutralising the electric charge of the inner layer of ions, is bound by adsorption forces and forms a so-called Stern layer. The remainder of the ions of the same sign are bound by electrostatic forces and form a so-called diffusion layer (also known as Gouy–Chapman layer). Their number decreases with increasing distance from the colloidal particle.[26]

During the relative motion of dispersed particles with the electric double layer against the dispersion medium, the Stern layer and part of the diffusion layer move with the particle while the rest of the diffusion layer moves with the fluid. A potential thus arises in the interface with the liquid, which is called the **electrokinetic potential** or ζ-potential (zeta-potential). Its size depends on the type of electrolyte and the ability to adsorb ions. The existence of electrical charge on the dispersed phase particles (the existence of electric double layer) significantly affects the stability of many dispersed systems. It is also associated with the phenomena that occur when one phase moves relative to another (in liquid–gas, liquid–liquid and liquid–solid systems) or with the behaviour of dispersed systems under an external electric potential gradient.

The four possible types of electrokinetic phenomena are streaming (current) potential (electric potential generated by fluid movement relative to another phase), sedimentation potential or Dorn phenomenon or Dorn effect (due to dispersed particles motion relative to the fluid caused by sedimentation) and electrophoresis and electro-osmosis (movement of two phases is caused by an external potential difference).

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[24] Light (electromagnetic waves) passing through homogeneous environments (e.g. through true solutions) causes polarisation of particles (molecules). The resulting dipoles emit light of the same wavelength and due to the interferences of secondary waves (Huygens' principle), the light spreads only in the direction of the primary light (incident) wave. In heterogeneous environments (e.g. in dispersions), this process leads to a different polarisation of dispersed phase particles and dispersion medium particles. The radiation is not compensated according to the Huygens' principle and is scattered in all directions. The light path through the dispersion system can be seen in a darkened room (Tyndall effect).

[25] Light scattering is expressed by the Rayleigh ratio $R_\theta = I_\theta.r^2/I_0(1 + \cos^2\theta) = \pi^2.\alpha^2.N/2\varepsilon_0.\lambda^4.V$, where I_θ = intensity of light scattered at an angle of θ, I_0 = intensity of incident light, λ = its wavelength, N = number of particles in volume (V) of dispersion, ε_0 = permittivity of a vacuum, $\alpha = \varepsilon_0.V/N.(\varepsilon_r - \varepsilon_r^0)$, where ε_r = relative permittivity of dispersion (for visible light $\varepsilon_r = n^2$), ε_r^0 = relative permittivity of pure solvent (for visible light $\varepsilon_r^0 = n_0^2$), n = dispersion refractive index and n_0 = refractive index of pure solvent. Light scattering measurement (or turbidity measurement, which is the total energy that scatters in all directions from the beam when the light passes through dispersions) can be used to determine the molecular weight and shape of dispersed phase molecules.

[26] The ratio of charges in the inner and outer layer can be calculated using Stern's equation: $-\sigma_1/\sigma_0 = I^{1/2}/k + I^{1/2}$, where σ_1 = charge of the inner layer of ions, σ_0 = charge of the outer layer of ions, I = solution ionic strength and k = constant. Diffusion layer thickness is given by the equation: $[\varepsilon_0.\varepsilon_r.k_B.T/e^2\Sigma(z_i^2.\nu_{i0})]$, where ε_0 = permittivity of vacuum, ε_r = relative permittivity of the medium, e = elementary charge, z_i = number of elementary charges, which the ion I carries, ν_{i0} = number of particles per volume unit, k_B = Boltzmann constant and T = absolute temperature.

Table 7.10 Water activity of selected foods.

Food	Water activity	Food	Water activity
Fresh meat and fish, milk, beer	0.99	Fruit jams, marmalade, jellies	0.75
Eggs, vegetables and fruits	0.98–0.97	Honey, dried fruits, caramel	0.7–0.6
Bread	0.97–0.95	Pasta products (such as noodles)	0.5
Some cheeses (such as Swiss cheese)	0.91	Spices (10% of water)	0.4
Fermented sausages, margarine, aged cheese	0.87–0.85	Biscuits	0.3
Sausages	0.85–0.82	Dried milk and instant coffee	0.2
Condensed milk, legumes (15% of water), flour	0.8	Sugar, dehydrated soups	0.1

7.9 Water activity

The amount of water present in foods has only a very vague relationship with their resistance to microbial attack and biochemical and chemical reactions, which occur in foods. A more important factor than the actual water content is its availability. Water availability is related to interactions of water with food components, with the bond strength of water bound by physical adsorption or chemisorption. Tightly bound water is less available than weakly bound water, which is again less available than free water. The measure of water availability is **water activity**. The water activity of a food is not the same thing as its moisture content.

All interactions of water in food (and also in solutions of electrolytes and non-electrolytes) result in a decrease in entropy, accompanied by a decrease of the vapour pressure of water. It can be inferred that the vapour pressure of water is related to water activity (a_w) by the following formula:

$$a_w = p_w/p_w^0$$

where p_w = partial pressure of water vapour above a solution of solids or liquids or above foods and p_w^0 = partial vapour pressure of pure water at the same temperature. Water activity values therefore range from 0 to 1.

When food is in equilibrium with the ambient air, the water activity of food is equal to the equilibrium relative humidity (φ; lowercase phi), which ranges from 0 to 100%:

$$a_w = \varphi/100$$

The water activity of some selected foods is given in Table 7.10. The water content of many foods and thus their activities vary according to humidity of the ambient air (and thus temperature), as there is a constant sorption or desorption of water. The term water activity should be used only for systems that are in thermodynamic equilibrium. Foods are often multicomponent multiphase systems and only if there is a thermodynamic equilibrium between all phases is the water activity in the whole system equal, which does not happen often. It frequently requires hours, weeks or takes even longer to achieve the equilibrium state. Many food systems may also be in a non-equilibrium metastabile state (e.g. sugar melts).

The water activity of a food increases with increasing temperature at constant moisture content.[27] An increase in temperature of 10 °C causes an increase in water activity value of the food of about 0.03 to 0.2 and may, for example, have a negative effect on the stability of packaged foods, where the water content in the system is constant: undesirable microorganisms may grow or some adverse reactions may occur.

Knowledge of water activity in foods and of the relative humidity of the ambient air allow us to predict under what circumstances the food, in contact with the air of a certain relative humidity, will dry up and when, on the contrary, the food will become moist. However, water activity tells us nothing about how the water is bound in food. If the water activity in food is higher than the ambient humidity, food loses water and dries up until equilibrium is established. In the equilibrium, the water activity in food is equal to the equilibrium relative humidity of the ambient air. On the other hand, if the water activity in food is lower than the relative humidity of the ambient air, food takes water from the ambient air and becomes moist. Prevention of adverse changes in water activity in food during production and storage (drying or wetting) and controlled removal of water by dehydration or drying (the purpose is to reduce weight and extend the food shelf life) is important in many food technologies.[28]

[27] Temperature dependence of water activity is described by the so-called Clausius–Clapeyron equation: $d\ln a_w/d(1/T) = -\Delta H/R$, where R = gas constant, T = absolute temperature and ΔH = integral adsorption heat. The dependence of the a_w logarithm on the temperature (or the $1/T$ value, respectively) in a certain temperature range is linear with the line slope $= -\Delta H/R$).

[28] Water activity in foods is usually determined from knowledge of the equilibrium relative humidity or can be measured using various hygrometers. In some foods it can be calculated from various theoretical and empirical models that take into account the food chemical composition, the content of electrolytes such as sodium chloride and non-electrolytes such as saccharose, respectively. Equations, varying in their levels of complication, are numerous, but their use is limited to certain commodities. One of the simple empirical equations for water activity calculation in jams has the form: $a_w = 1/(1 + 0.27n)$, where n = number of moles of saccharose per 100 g of jam. The following equation was used to calculate water activity in confectionery $a_w = 1.04–0.1\sum s_i.c_i + 0.0045\sum(s_i.c_i)^2$, where c_i = concentration of non-electrolyte (component i), s_i = its equivalent versus saccharose (e.g. the value is 1.0 for saccharose and lactose and 1.3 for invert sugar and gelatine).

Table 7.11 Requirements of selected microorganisms to a minimum water activity.

Bacteria	Yeasts	Molds	a_w	Bacteria	Yeasts	Molds	a_w
Pseudomonas			0.96		*Debaryomyces*		0.87
Salmonela			0.95	*Staphylococcus*			0.86
Escherichia							
Bacillus							
Clostridium							
Salmonela			0.94			*Penicillium*	0.85
Escherichia							
Bacillus							
Clostridium							
		Rhizopus	0.93			*Aspergillus*	0.65
		Mucor					
	Rhodotorula		0.92		*Zygosaccharomyces*		0.62
	Pichia						
Micrococcus	*Saccharomyces*		0.90			*Xeromyces*	0.60
	Hansenula						
	Candida	*Cladosporium*	0.88				
	Torulopsis						

7.9.1 Water activity and microorganisms

Knowledge of water activity is also important to assess the potential susceptibility of foods to spoilage by microorganisms (Table 7.11). Most microorganisms grow well at a_w ranging from 0.91 to 0.99. Common bacteria need at least a water activity value of 0.94 for their growth, yeasts 0.90 and fungi around 0.75 (Figure 7.40). Bacteria *Clostridium botulinum* will grow and produce the deadly botulism toxin if the water activity is above 0.85 and the pH value is above 4.6. Most halophilic bacteria and mycotoxigenic aspergilli can reproduce in media where the water activity value is at least 0.75. Xerophilous fungi grow at water activity of 0.60 and osmophilic yeasts are adapted to the environment of about the same water activity (0.65 or 0.60), which causes problems even in otherwise stable foods such as dried fruits and honey. This property of microorganisms is related to their chemical composition, the presence of polyols (glycerol and ribitol) in fungi and the higher content of proline and glutamic acid in bacteria.

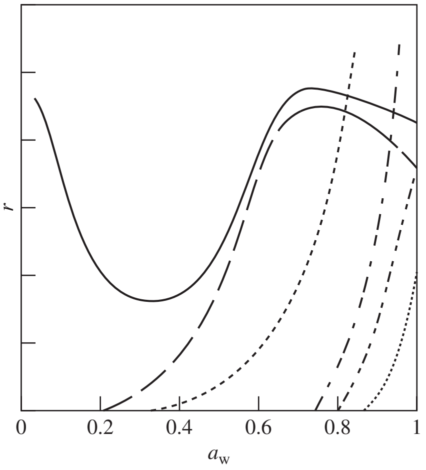

Figure 7.40 Influence of water activity on microorganisms and important reactions in foods: r = relative reaction rate, a_w = water activity, —·—·—· = molds, —· · —·· = yeasts, ~ · ~ · ~ = bacteria, ·········· = enzymatic activity, — — — = Maillard reaction, —— = lipid autoxidation.

7.9.2 Water activity and reactions

In addition to microbial activity, water activity also affects many important enzymatic and non-enzymatic reactions that proceed in foods during processing and storage (Figure 7.40).

For example, the enzymatic hydrolysis of lipids in dried meat is a negligible process up to the point where water activity decreases below 0.4. Although lipid autoxidation rate decreases with decreasing water activity (up to about 0.3–0.5, because a certain amount of water inhibits the decomposition of lipid hydroperoxides), it then increases again with decreasing water activity with a maximum reaction rate in water activity close to zero. This odd behaviour

is explained by the removal of water from the hydrophilic sites of the material, therefore more lipid molecules are thus exposed to atmospheric oxygen.

The Maillard reaction rate increases with gradually decreasing water activity and the maximum reaction rate is at a water activity of about 0.7–0.8, which is attributed to the increase in the concentration of reactants (amino compounds and reducing sugars). With further decrease of water activity, the reaction rate decreases. The Maillard reaction does not proceed at all when the water activity decreases to less than 0.2–0.3, because the mobility of reactants is too low.[29]

7.9.3 Water activity and food organoleptic properties

Water activity is also related to the organoleptic properties of foods, especially foods with low or medium water content. At water activity values 0.35–0.50, some foods show desirable organoleptic properties such as crispness and crunchiness (e.g. potato crisps and extruded cereal products) that are mostly lost when the food becomes moist. In this range of water activities, instant beverages also become moist and, as a result, undesirable changes may occur, such as crystallisation of amorphous sugars, wetting and dissolution of their crystals, stickiness and colour changes.

7.9.4 Free and bound water

Water contained in foods was previously classified as **bound water** and **free water**, or as **movable water** and **immobilised water**. Such a classification takes into account the strength of water bonds in food and is related to how easily water can be mechanically removed from the food (e.g. by pressing) or by physical processes (such as drying). These terms are very vague, however, often poorly understood and mostly incorrectly used. According to some opinions, all the water contained in foods can be classified as bound water. In common foods with high water content (more than 90%), **bound water** exists at water activities ranging from 0.0 to 0.7, and is often divided into the following categories.

- The very tightly bound water is present as **constitutional water**, which is an integral part of hydrates (<0.03% of total water). About 0–1% of the total water is found in the immediate vicinity of molecules of organic substances in foods, and has the following attributes: it has a lower mobility in comparison with the bulk water in the same food, is bound mainly by chemisorption, particularly by the hydrophilic polar groups of proteins and polysaccharides (by water–ion and water–dipole associations), it can form a continuous layer surrounding hydrophilic molecules (proteins and polysaccharides) or their hydrophilic sites, it does not have the function of a solvent and does not freeze even at –40 °C. This proportion of water (generally 0.5 ± 0.4% of the total water) is referred to as **vicinal water**. At this water content, which roughly corresponds to the monomolecular layer, no chemical reactions proceed. For example, the water content corresponding to the monomolecular layer is reportedly 11% in gelatine and starches, 6% in dried potatoes, 3% in whey powder and in crystalline saccharose 0.4% of the total water content.

- Another proportion of bound water (3 ± 2% of the total water content) exists at water activities ranging from 0.2 to 0.7. This water occupies the remaining first-layer sites and forms several layers around the monomolecular layer. In these layers mutual hydrogen bonds between water molecules already dominate, but there are also interactions between water molecules and ions or dipoles. Some water molecules penetrate into the capillary pores in the food structures by physical sorption. This water has the limited function of a solvent and the main proportion of this water does not freeze at –40 °C. The boundary between the first and second category water is the value of water activity of about 0.25. This water is known as **multilayer water**.

- The water activities ranging from 0.7 to 1.0 amounts approximately 90–96% of the total water content. Water occurs more or less as **free water**, which has similar properties as water in diluted salt solutions, or has all the attributes of pure water. It mainly acts as a solvent of inorganic and organic substances. This water does not come out of foods freely, as it is bound exclusively by physical sorption (capillary forces), but can be released from foods using suitably small forces (such as the gravitation force). The boundary between the water of the second and third category is at a water activity value of around 0.7 and higher. Sometimes, this category of water is known as **condensed water**; two types are also recognised, these being **trapped water** and **free water**.

Classification of water is also possible on the basis of thermodynamic properties. The binding enthalpy of water of the first category (vicinal water) is about −4 to −6 kJ/mol, of the second category of water (multilayer water) approximately 1–3 kJ/mol and of the third category of water (condensed water) around –0.3 kJ/mol.

7.9.5 Sorption isotherms

The relationship between water content in food (or equilibrium relative humidity of ambient air) and water activity is not simple. It is best described by the **sorption isotherm** of a particular food, which is the dependence of its water content on water activity.[30]

[29]In foods with low or medium water content the limiting factor is mobility and especially diffusivity of the reacting molecules. For example, the rate of reaction A + B = C is normally given by the rate constant (k). If the reaction rate is limited by diffusion, the rate constant $k'=k/1 + (k/\alpha.D)$, where α=constant independent of temperature and (D) is diffusion coefficient, $D = D_A + D_B$ (k and D are dependent on temperature). According to the Stokes–Einstein equation (D) value depends on the hydrodynamic radii of reactants and the viscosity of environment and therefore depends on water content. The influence of temperature on reaction rate is a function of the environment viscosity (η) in the diffusion-controlled reactions as it is given, according to the temperature at which the reaction takes place, by the Arrhenius equation or WLF equation.

[30]Physical chemistry phenomena at the interface describe by the name adsorption isotherm the relationship between the amount of substance present in the

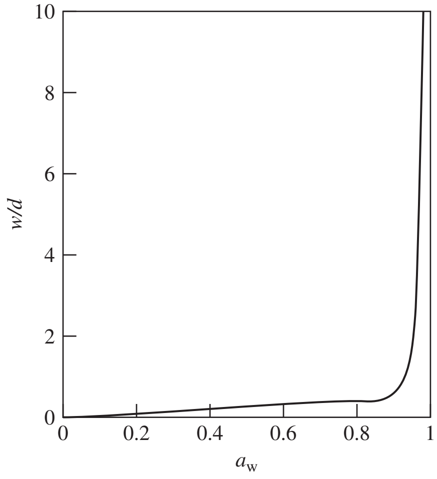

Figure 7.41 General sorption isotherm of food in a wide range of water contents: *w/d* = grams of water per gram of dry matter, a_w = water activity.

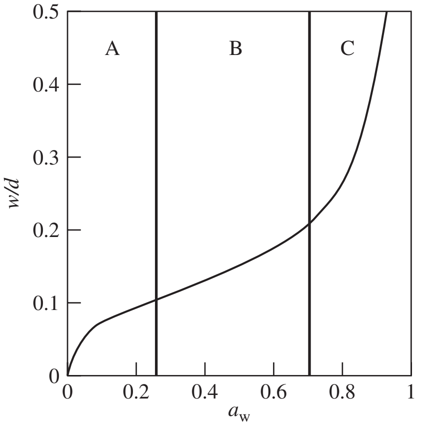

Figure 7.42 Detail of general sorption isotherm: *w/d* = grams of water per gram of dry matter, a_w = water activity, A = vicinal water, B = multilayer water, C = condensed water.

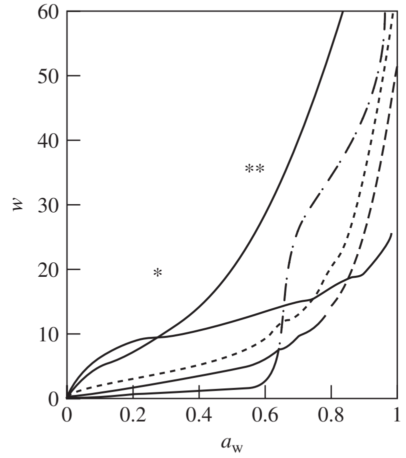

Figure 7.43 Various types of sorption isotherms: *w* = water amount (%), a_w = water activity, —— = dry hydrolysed vegetable protein, ┈┈┈ = dry whey, –·–·–· = ground roasted coffee, – – – = wheat flour, –· · –· · = saccharose.

The sorption isotherm of a hypothetical food with high water content is shown in Figure 7.41, while the same isotherm over a narrow range of water contents and indicating the existence of vicinal water, multilayer water and condensed water is shown in Figure 7.42.

Various foods have different sorption isotherm shapes (Figure 7.43) depending on their physical microstructure and macrostructure, qualitative and quantitative chemical composition and distribution of chemical components. Attempts have been made to explain the shape of isotherms of food of different chemical composition, but their importance lies more in practical application than in theoretical interpretation. From the sorption isotherms of dried acid protein hydrolysate (Figure 7.43), which is strongly hygroscopic, it can be seen that even a very small change in water activity (or the humidity of the ambient air) causes a significant increase in water content in the hydrolysate. In the point marked *, the visible changes in the hydrolysate water content are beginning, as originally powdery particles stick together and change their colour from light brown to dark brown. At the point marked **, the hydrolysates become a viscous sticky mass.[31] An example of a moderately hygroscopic material is saccharose, and a non-hygroscopic material is ground coffee.

The sorption isotherms of foods are only valid at the particular temperature at which the results were obtained. Many materials (such as fibrous proteins, cellulose and most foods) have different water activity at the same water content, depending on whether the system absorbs or desorbs water. The corresponding isotherms then have a different shape, and therefore the absorption isotherms and desorption isotherms are recognised. The phenomenon is called a **hysteresis** (Figure 7.44). In general, the water activity is higher when the food absorbs water. If it loses water, then the water activity

surface layer (the surface excess) and the change of surface tension with concentration (Gibbs adsorption isotherm). In chemistry the Langmuir adsorption isotherm is often used, which is a very simplified description of adsorption. It is sometimes similarly applied to describe the sorption of water in foods. More often, however, the BET isotherm is used, which provides information on the nature and extent of interactions of water in foods. The importance lies in the fact that it is possible to calculate the amount of water corresponding to the monomolecular layer (v_m) from the linearised equation: $a_w/(1-a_w).v = a_w.(c-1)/v_m.c + 1/v_m.c$ (a_w = water activity, V = volume of water adsorbed in 1 g of dry food, c = constant related to the adsorption heat). This allows estimation of the water content in the food at which the dry product has maximal stability.

[31] Agglomeration of particles of initially powdery material, between which the liquid forms a film that agglutinates them, depends on surface tension and viscosity of the film. This agglomeration can be, for example, described by the equation: $t = 2R\eta/3\gamma(r/R)^2$, where t = time of contact of material particles, R = initial radius of particle, r = radius of liquid film between particles, η = film viscosity and γ = film surface tension. The critical viscosity range is 1×10^5 to 1×10^8 Pa s.

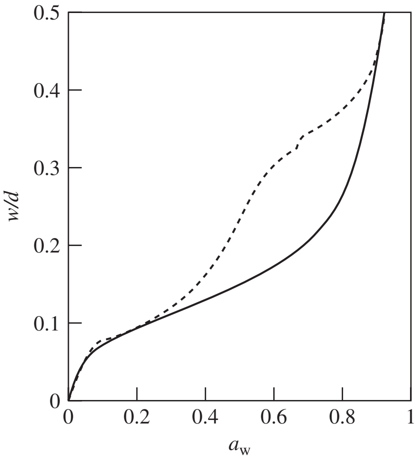

Figure 7.44 Hysteresis during water absorption and desorption: w/d = grams of water per gram of dry matter, a_w = water activity, —— = absorption, − − − − = desorption.

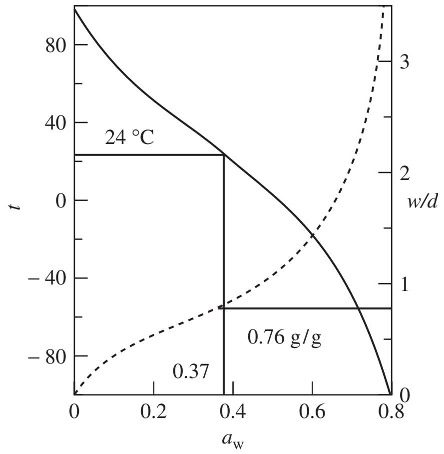

Figure 7.45 Sorption isotherm of powdered milk in association with glass transition temperature: t = temperature (°C), w/d = grams of water per gram of dry matter, a_w = water activity, − − − − = sorption isotherm —— = glass transition temperature (T_g in °C).

is lower. The physical nature of the hysteresis appears to be related to capillary phenomena in foods and their eventual collapse. The practical significance of the existence of a hysteresis is that, when adjusting the water content in food to a water activity value at which a certain reaction does not take place, it is better to reach this value by absorption, because the material then binds water more tightly than the material of the same activity that has been achieved by desorption.

The importance of sorption isotherms is generally in the evaluation of the water content in food at which the adverse effects on the food quality can be minimised. This is usually the moment when other layers are formed around the monomolecular layer of water (vicinal water), which is the instant when the multilayer water arises. Most adverse events in storage of foods with medium and low water content, such as crystallisation of amorphous sugars (e.g. lactose in powdered or condensed milk), agglomeration of powder materials, stickiness or re-crystallisation of water and formation of large crystals in frozen products (e.g. in ice cream) relates to water content, its activity and storage temperature, and are therefore closely related to the glass transition temperature of the material.

Figure 7.45 illustrates, together with the sorption isotherm, the glass transition temperature of dried milk (virtually identical to the glass transition temperature of lactose), depending on the activity of water. This dependence allows the prediction of the influence of water activity (a_w) or equilibrium relative humidity (φ) on glass transition temperature (T_g) and allows evaluation of product stability or localisation of critical values of water activity. The critical value at 24 °C is the value $a_w = 0.37$ at which the (T_g) value is the same as the storage temperature (24 °C). Under such conditions, the stored dried milk contains 0.76 g of water in 1 g of dry matter, which leads to agglomeration of particles of milk and also to the crystallisation of amorphous lactose, which causes a sandy consistency of the product. The rate of these changes is determined by the difference between storage and glass transition temperatures ($T - T_g$). Minor changes occur during storage at lower temperatures (at $T < T_g$).

8

Flavour-active Compounds

8.1 Introduction

The most important psychological factor in human nutrition is the sensory quality of food, which significantly affects the type and quantity of food consumed and its utility. Sensory quality of food depends on the presence of **sensory-active substances** that are perceived by chemical sensors and which determine the **sensory value** of foods by inducing **olfactory**, **gustative** and other sensations (such as visual, haptic or tactile and auditorial; see Chapter 1). The **organoleptic properties** of foods are usually more important to the consumer than other attributes, such as the vitamin content, because this information is observed first, and thus contributes significantly to the overall impression of the food raw materials and foods. Many foods and dishes are therefore aromatised, flavoured, coloured or their texture is improved, while many others are consumed solely for their sensory value for example, various delicatessen items (delicacies, gourmet or fine foods), such as caviar or truffles.

Olfactory sensation results when olfactory receptors in the nose are stimulated by a particular substance in gaseous form called an **odorant**, which is capable of being translated into the subjective responses of neural brain stimulation that we term **odour**, **smell, aroma**, **flavour** or **scent**. Gustatory sensation that determines the **taste** is a sensation elicited by substances acting on taste receptors in taste cells in the mouth.

Depending on their origin, the odour- and taste-active substances can be divided into two basic groups:

- Those that are already present in food raw materials or foods, as products of the secondary metabolism. These secondary metabolites are produced by intracellular processes and their quality and quantity depend mainly on the genetic predisposition of the organism (plant or animal species). In plants, animals and other organisms, there is a variability within certain limits that is caused by some external factors. In plants, it may be location, age, degree of maturity (vegetative stage), harvest time, various environmental factors (the amount of moisture, nutrients, temperature and light) and conditions during post-harvest storage. These flavour-active substances are also known as **primary flavour-active substances**.

- Many odour- and taste-active substances occur in foods in a bound, sensorially inactive form, mainly as glycosides or esters. These compounds release the sensorially active substances by the action of enzymes, glycosidases and hydrolases of carboxylic acid esters, for example, during tissue disintegration. Sensorially active substances also result during storage and processing of food as products of enzymatic and non-enzymatic reactions from proteins, carbohydrates and lipids (the primary products of the metabolism), or from other chemical substances, such as vitamins and various pigments. Fermentation processes and thermal processing of foods (during cooking, baking, frying, smoking and drying) are the main processes in which these substances are formed from their precursors. Autoxidation, the Maillard reaction (non-enzymatic browning reactions) and enzymatic browning reactions are the major reactions that lead to the formation of these flavour-active substances during storage, industrial and culinary processing of foods. Sensorially active substances generated during these processes are often referred to as **secondary flavour-active substances**.

The resulting sensations of smell and taste, but also the sensation of colour and texture of food, are due to substances that are usually complex mixtures of several compounds. Odoriferous (odour-active) substances (odorants) are compounds that act on the olfactory receptors and give the impression of odour. They can also affect the taste receptors, in which case they are also taste-active substances. An example of such a compound is acetic acid, which has a penetrating odour resembling vinegar, and a sharp sour taste. Odoriferous substances are predominantly low-polar or non-polar (water insoluble to slightly soluble) compounds, producing a wide variety of sensory perceptions. Taste substances are those that act on taste receptors and give the impression of taste in the mouth.

The Chemistry of Food, First Edition. Jan Velíšek.
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Gustatory sensation includes five basic tastes: **sweet**, **salty**, **sour**, **bitter** and **umami**.

Common sweet substances are monosaccharides and disaccharides, such as saccharose (sucrose, table sugar), while the best known salty substance is sodium chloride (table salt). A sour taste is found in the previously mentioned acetic acid found in vinegar and pickled vegetables, and bitter tasting compounds are caffeine in coffee and quinine in tonic water. The umami taste was officially recognised as a legitimate fifth taste in the 1980s because it complements the conventional taste categories of sweet, sour, salty and bitter. Opinions vary as to the precise translation of the word umami, but the best approximation is something like 'savoury deliciousness'.

The oral cavity also responds to other sensations, such as those described as astringent taste, pungent (hot, burning) taste or some others. There is an increasingly popular belief that taste is more complicated than was originally believed as it is related to a combination of sensations such as smell, colour, texture and the sound of foods when chewing, and even to the emotional circumstances of the consumer when eating.

Taste-active substances are usually water-soluble polar and non-volatile substances. Some taste-active substances may additionally be odour-active substances, but not necessarily. The complex (uniform) sensory perception of taste and smell is called flavour; a strong pleasant smell, usually from food or drink is called aroma, and aromatic food or drink is that which has a pleasant smell. However, the term 'aromatic substance' has a completely different meaning in organic chemistry. An aromatic substance, put simply, is a benzene derivative. The unwanted, altered, modified, unnatural or unpleasant odour, taste and flavour are called **off-odour** and **off-taste** or **off-flavour**, respectively.

Substances influencing **colour** (such as dyes and pigments) are also important sensory-active substances present in foods. They determine not only the characteristic colour of the food, but also affect the taste threshold concentrations of substances and the ability to identify smells. Food that has a satisfactory nutritional and hygienic-toxicological quality or excellent odour, taste and texture will still not be accepted by consumers unless the characteristic colour corresponds to the standard product.

The properties included under the heading **texture** are found mainly in macromolecular food components, especially proteins and polysaccharides, as well as products of their interactions and associations with other food ingredients, first and foremost with water. Texture implies those characteristics of foods that cause tactile or haptic sensations registered by receptors in the oral cavity. Touch by hands is very important. Auditorial perceptions such as crispness are related to a range of textural characteristics. Geometric attributes of texture that simultaneously cause visual and haptic sensations, often referred to as shape and appearance (such as particle size or size of the whole food), are closely related to food colour. The term **consistency** describes the texture aspects related to physical (mechanical) properties of food, which are also called rheological properties (see Section 7.8.4.1).

This relatively extensive chapter is devoted to components that affect food odour and taste. Also included are a number of organic compounds of plant origin, which, although not affecting the odour or taste of foods, are structurally similar to the sensory-active substances and also have important biological effects, for which they are at the forefront of medicine and pharmacy. In the first section, the aromatic compounds are classified into their common groups, so hydrocarbons, and their oxidation products that include alcohols, aldehydes, ketones, carboxylic acids and functional derivatives of carboxylic acids (esters, lactones and nitriles) are described. Other groups of organic compounds discussed are phenols, sulfur- and nitrogen-containing compounds, including heterocyclic compounds. Each section presents the structure of individual compounds, their properties, occurrence and important reactions that occur in food. Another part of the chapter is devoted to odour-active substances in individual food commodities (meat and meat products, poultry, fish, milk and dairy products, eggs, cereal products, fruits, vegetables, alcoholic beverages, tea, coffee, cacao and chocolate, nuts, honey, mushrooms and spices). Next, the importance of aromatic compounds in the diet, their organoleptic properties, biological effects, production and use are dealt with. The last part is devoted to substances that affect the taste of foods. These substances are categorised into groups according to the basic and also other tastes: sweet, salty, sour, bitter, astringent and pungent (sharp, burning), and commodities in which they are found. The structure of these compounds, their occurrence, properties, quality of taste perception, reactions, significance in nutrition, physiology and technology and practical use are all described.

8.2 Odour-active substances

The aroma of food is often a very complex phenomenon caused by a large number of odour-active substances. The total number of odorants identified in foods is estimated to be about 10 000, and each food commonly contains several hundred different aromatic compounds. Some compounds are not involved in the characteristic odour of a particular food at all, some contribute very little, but others are of fundamental importance for various reasons, such as their odour character, low threshold concentration of perception or high concentration. The resulting odour impression is thus caused by several compounds. The intensity and quality of odour, however, depends not only on the odoriferous substances present, but also on other food components, especially proteins, carbohydrates and lipids, with which the odorants interact. These non-bonding interactions influence the concentration of aromatic substances in the gaseous phase. Typical examples are allyl mercaptan, diallyl disulfide, methyl mercaptan and allyl methyl sulfide, responsible for the smell of crushed garlic and the so-called garlic breath, while the smells of oranges and roasted coffee result from about two dozen different compounds. In only a limited number of cases are the typical smells associated with a single substance or a few compounds, which are called **key components**. An example is vanillin, which is practically the only substance responsible for the typical smell of dry vanilla beans. Some other examples are listed in Table 8.1.

Volatile flavour-active components of foods include virtually all groups of organic compounds, hydrocarbons and their oxygen derivatives (such as alcohols, aldehydes, acetals, ketones, carboxylic

Table 8.1 Characteristic (key) odorous components of foods.

Compound[a]	Descriptor	Occurrence
(−)-(*R*)-Oct-1-en-3-ol	Mushroom-like	Mushrooms, molds
(−)-Geosmin	Earthy	Red (garden, table) beet root
Anethole	Anise-like	Anise seeds
Cinnamaldehyde	Cinnamon-like	Cinnamon bark
Vanillin	Vanilla-like	Dry vanilla beans
Eugenol	Clove-like	Clove plant fruits
(*E*)-Citral (geranial, citral a) and (*Z*)-citral (neral, citral b)	Lemon-like	Lemons
(2*E*,6*Z*)-Nona-2,6-dienal	Cucumber-like	Fresh cucumbers
Benzaldehyde	Bitter almond	Bitter almonds, sour cherries
(+)-(2*E*,5*S*)- and (−)-(2*E*,5*R*)-5-methylhept-2-en-4-one[b]	Hazelnut-like	Roasted hazelnuts
4-(4-Hydroxyphenyl)butan-2-one (raspberry ketone)	Raspberry-like	Raspberries
(+)-(*S*)-Carvone	Caraway-like	Caraway and dill seeds
(2*E*,4*Z*)-Ethyl deca-2,4-dienoate	Pear-like	Pears
5-Ethyl-3-hydroxy-4-methyl-5*H*-furan-2-one (abhexon)	Hydrolysate-like	Acid protein hydrolysates
(+)-(*R*)-*p*-Menth-1-ene-8-thiol	Grapefruit-like	Grapefruits
Diallyl disulfide	Garlic-like	Garlic
Maltol and isomaltol	Caramel-like	Caramel
2-Acetyl-1-pyrroline	Crust-like	Bread crust, aromatic rice
2-Isobutylthiazole	Tomato-like	Tomato leaves, fresh fruits

[a] The structures of compounds are given further in the text.
[b] Trade name of the mixture of (*E*)-isomers is filbertone.

acids, their esters and other derivatives), amino derivatives (amines, nitrogen heterocyclic compounds), sulfur derivatives (such as thiols, sulfides and sulfur heterocyclic compounds) and many more. Particularly important primary flavour-active substances are **terpenes** and **terpenoids**. Terpenes are naturally occurring hydrocarbons derived biosynthetically from units of isoprene (2-methylbuta-1,3-diene) linked together head to tail (or, more rarely, head to head) to form linear chains, or they may be arranged to form rings. Terpenes modified chemically, such as by oxidation or rearrangement of the carbon skeleton, are terpenoids, sometimes referred to as **isoprenoids**. Terpenoids are the largest class of plant secondary metabolites with over 20 000 known compounds, produced primarily by a wide variety of plants, where they serve a range of different functions in basic and specialised metabolism. Depending on the number of isoprene units, isoprenoids acting as odour-active substances are divided into:

- **hemiterpenes** and **hemiterpenoids** (C_5 compounds containing one isoprene unit)
- **monoterpenes** and **monoterpenoids** (C_{10} compounds containing two isoprene units)
- **sesquiterpenes** and **sesquiterpenoids** (C_{15} compounds containing three isoprene units).

The main volatile components in essential oils are monoterpenes and monoterpenoids. Historically they have been used in the food, perfume and pharmaceutical industries because of their culinary, fragrant and antimicrobial properties. In fruits and vegetables, herbs and spices, and in wines, they express a wide spectrum of odours, most of which are perceived as very pleasant. Sesquiterpenes and sesquiterpenoids are among the most widely occurring odorants. Numerous sesquiterpenic hydrocarbons, alcohols and derived metabolites found in plant essential oils are highly valued for their desirable flavour characteristics. They also display a broad range of physiological properties, including antibiotic, antiviral, antifungal, antitumour and hormonal activities. Synthetic variations and derivatives of natural terpenes and terpenoids are additionally used to greatly expand the variety of aromas used in perfumery and flavours used in food additives.

Secondary flavour-active substances arise in particular:

- as metabolic products of microorganisms in fermentation processes

- by oxidation and degradation of labile constituents (such as lipids and carotenoids)
- in thermal processes, especially from proteins and carbohydrates in the Maillard reaction.

For completeness, this chapter also includes some non-volatile substances that do not have the potency of odour-active compounds. However, these substances may frequently be precursors of volatile compounds or have other important properties.

8.2.1 Hydrocarbons

Hydrocarbons are common components of many foods often occurring as constituents of essential oils and food lipids. They are either natural components of food raw materials and foods (primary substances) or form during food processing and storage by enzymatic and chemical reactions as secondary substances (e.g. as lipid oxidation products and degradation products of carotenoids). Some hydrocarbons may be present in food as endogenous or exogenous contaminants (e.g. polycyclic aromatic hydrocarbons).

For food flavouring, hydrocarbons are used relatively rarely, but they are used as the starting material for the synthesis of other volatiles. Some aliphatic hydrocarbons (such as hexane) or in a mixture (e.g. petroleum ether and gasoline) are also used as solvents in the flavour and fragrance industry and in oleochemistry. Residues of these hydrocarbons may therefore be present in essential oils, oilseed meals and other materials obtained by extraction with hydrocarbons.

8.2.1.1 Classification, structure, terminology and occurrence

Depending on their structure, hydrocarbons occurring in foods can be divided into three basic groups: aliphatic, alicyclic and aromatic hydrocarbons. The most important flavour-active substances (in addition to natural dyes) are terpenic hydrocarbons (terpenes).

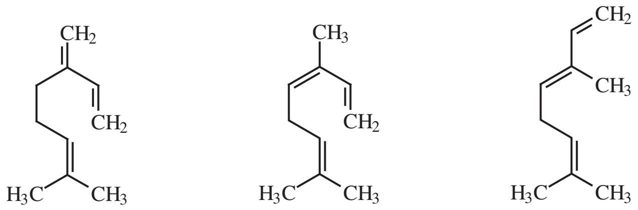
8.2.1.1.1 Terpenic hydrocarbons

Compounds with the formula $(C_5H_8)_n$, which includes monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$) can be active as odorous substances. Higher terpenic hydrocarbons, starting from diterpenes ($C_{20}H_{32}$), are indifferent as flavouring materials, but may act as taste-active substances or precursors of flavour-active substances. Terpenic hydrocarbons occur in almost all fruits, vegetables and spices. For example, about 90–99% of orange fruit volatiles and about 80% of carrot root volatiles are terpenic hydrocarbons. About 70–80% of black pepper volatiles are monoterpenic hydrocarbons and 20–30% are sesquiterpenic hydrocarbons. However, terpenic hydrocarbons are usually not very important compounds in defining the typical aroma of food commodities. More important are various monoterpenoids and sesquiterpenoids, alcohols, aldehydes, ketones, esters and other compounds. In pepper, for example, an important compound with peppery and spicy flavour is sesquiterpenoid (−)-rotundone (see **8-200**, later). About 1000 monoterpenoids, more than 300 distinct sesquiterpene carbon skeletons and more than 7000 oxidised or otherwise modified sesquiterpenic derivatives have been identified in nature. Most of these terpenoids are optically active compounds. Individual enantiomers and diastereoisomers may occur in different organisms or just in a single organism, but often they are as mixtures.

Terpenes are synthesised in organisms by complex mechanisms from isoprene units, isopentenyl phosphate and dimethylallyl diphosphate (pyrophosphate). The first product is geranyl diphosphate, which is the universal precursor of monoterpenoids. The reaction of geranyl diphosphate with another molecule of isopentenyl diphosphate yields farnesyl diphosphate, which is the precursor of sesquiterpenoids. Further reaction with isopentenyl diphosphate gives geranylgeranyl diphosphate, the precursor of diterpenoids (see Figure 3.10).

Monoterpenes

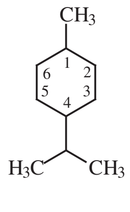
Monoterpenic hydrocarbons found in foods are linear (acyclic), monocyclic, bicyclic and tricyclic compounds. Linear monoterpenes are mainly present in fruits and essential oils. Examples of common hydrocarbons are myrcene (β-myrcene, **8-1**) and ocimene (β-ocimene, **8-1**), which occurs in two stereoisomers, as *trans*-isomer (*E*)-ocimene and as *cis*-isomer (*Z*)-ocimene.



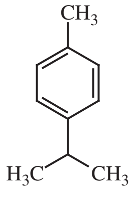
myrcene (*Z*)-β-ocimene (*E*)-β-ocimene

8-1, linear monoterpenic hydrocarbons

Monocyclic monoterpenic hydrocarbons are derived predominantly from the optically active hydrocarbon 4-isopropyl-1-methylcyclohexane, known as *p*-menthane (**8-2**). An exception is *p*-cymene also known as cymene (1-isopropyl-4-methylbenzene, **8-3**), which is an aromatic hydrocarbon. Cymene is a common component of many essential oils, especially the essential oils of cumin (the seed of the herb *Cuminum cyminum* of the parsley family Apiaceae) and common thyme (*Thymus vulgaris*, from the mint family Lamiaceae) listed in Table 8.32 (see later).

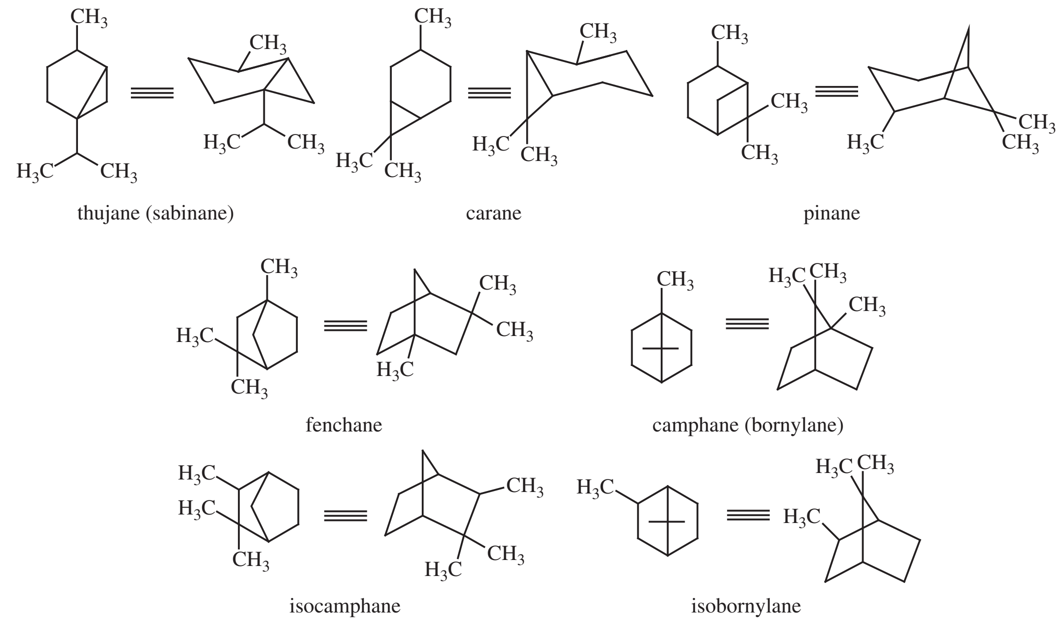


8-2, *p*-menthane



8-3, *p*-cymene

Bicyclic hydrocarbons can be divided into seven structural types. These systems are combinations of three- and five-membered rings



8-4, bicyclic monoterpenic hydrocarbons

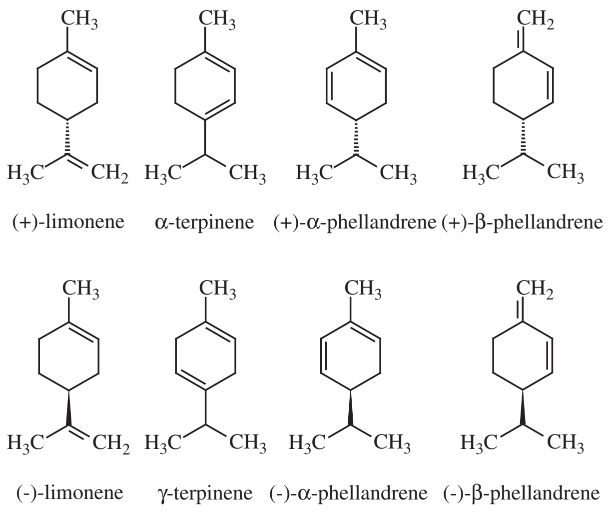
(thujane, also known as sabinane), three- and six-membered rings (carane), six- and four-membered rings (pinane) or two five-membered rings (fenchane, camphane, also known as bornylane, and isocamphane and isobornylane) (**8-4**).

The most important compounds are menthadienes, derived from *p*-menthane, which differ in the positions of the double bonds (**8-5**). A common hydrocarbon is limonene (*p*-mentha-1,8-diene), which typically occurs in many essential oils (such as essential oils of citruses, mints and conifers) and turpentine. For example, the (+)-limonene isomer is the major component (>90%) of essential oils of citrus peels, (−)-limonene is a component of essential oils of different types of mint (*Mentha* spp., Lamiaceae) and conifers. Racemate, which is trivially known as dipentene, occurs in many essential oils. Other important menthadienes include α-terpinene (*p*-mentha-1,3-diene), γ-terpinene (*p*-mentha-1,4-diene), α-phellandrene (*p*-mentha-1,5-diene) and β-phellandrene, also known as *p*-mentha-1(7),2-diene.

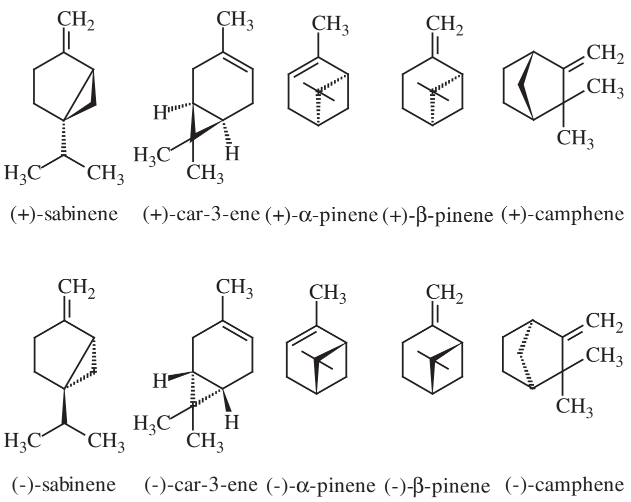
A number of bicyclic hydrocarbons are present in turpentine, and also as components of fruits, vegetables and spices (**8-6**). A frequently occurring compound is sabinene, also known as thujene or 4(10)-thujene (which is found in higher concentrations in the essential oil of black pepper), car-3-ene (Δ^3-carene), α-pinene (2-pinene), β-pinene, also known as 2(10)-pinene, nopinene or pseudopinene and camphene. Trivial and systematic names of the main compounds are listed in Table 8.2.

Sesquiterpenes

About 300 different basic structures are found in nature, from which sesquiterpenes and sesquiterpenoids are derived. Often



8-5, cyclic monoterpenic hydrocarbons menthadienes

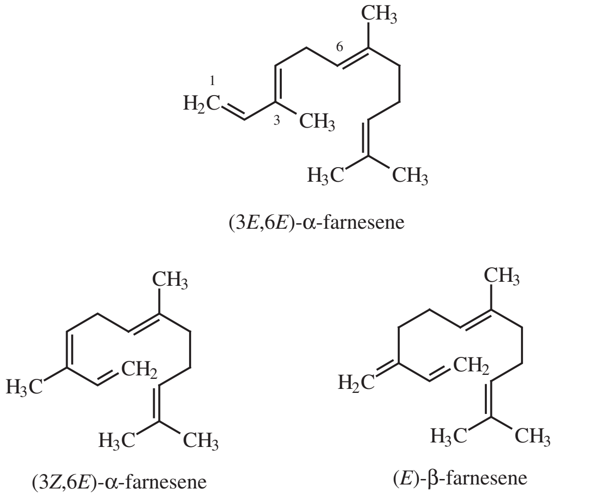


8-6, bicyclic monoterpenic hydrocarbons

Table 8.2 Trivial and systematic names of some monoterpenic hydrocarbons.

Trivial name	Systematic name (IUPAC)
(+)-α-Phellandrene	(*S*)-2-Methyl-5-propan-2-ylcyclohexa-1,3-diene
(+)-β-Phellandrene	(*S*)-3-Methylene-6-propan-2-ylcyclohex-1-ene
(+)-Camphene	(1*R*,4*S*)-6,6-Dimethyl-5-methylidenebicyclo[2.2.1]heptane
(+)-Car-3-ene	(1*S*,6*R*)-3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene
(+)-Limonene	(*R*)-1-Methyl-4-prop-1-en-2-ylcyclohex-1-ene
Myrcene	7-Methyl-3-methyleneocta-1,6-diene
(*Z*)-β-Ocimene	(*Z*)-3,7-Dimethylocta-1,3,6-triene
(*E*)-β-Ocimene	(*E*)-3,7-Dimethylocta-1,3,6-triene
(+)-α-Pinene	(1*R*,5*R*)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene
(+)-β-Pinene	(1*R*,5*R*)-6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane
(+)-Sabinene	(*R*)-4-Methylene-1-propan-2-ylbicyclo[3.1.0]hexane
α-Terpinene	1-Methyl-4-propan-2-ylcyclohexa-1,3-diene
γ-Terpinene	1-Methyl-4-propan-2-ylcyclohexa-1,4-diene

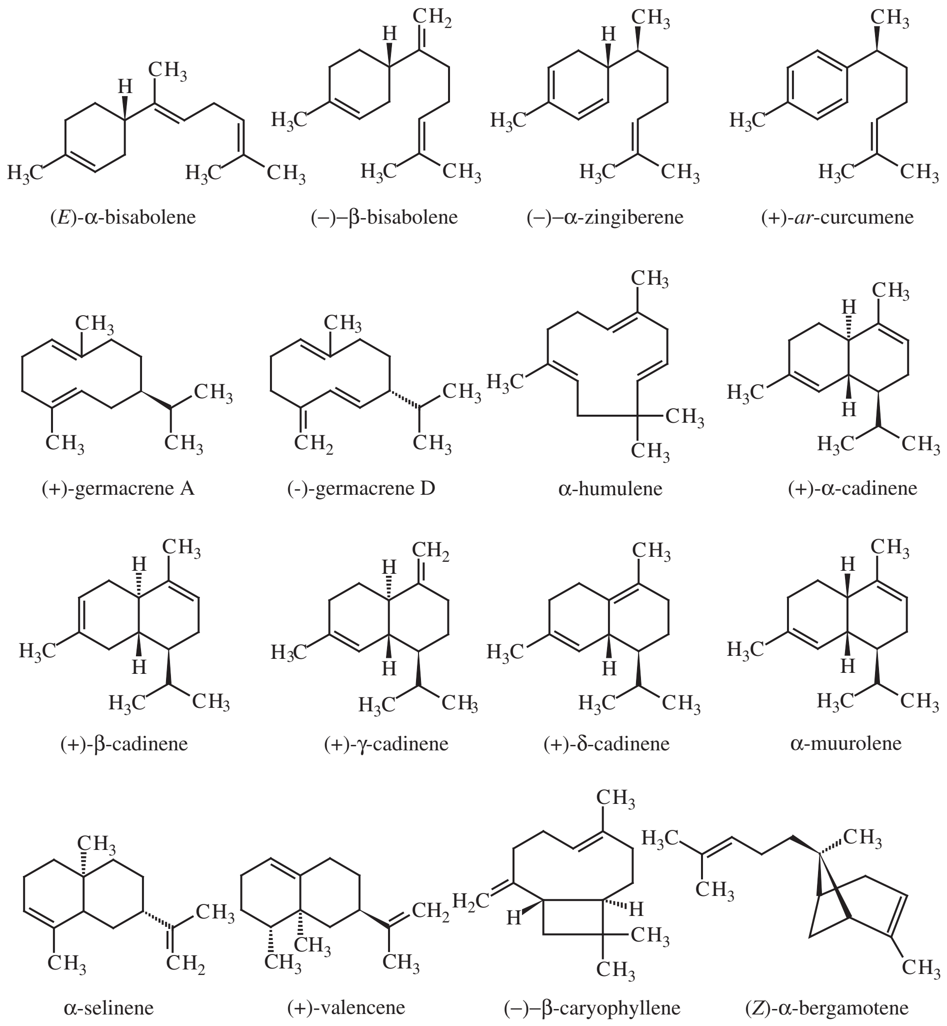
present in foods are stereoisomeric acyclic sesquiterpenic hydrocarbons called farnesenes, which include α-farnesene and β-farnesene. α-Farnesene exists as four stereoisomers that differ in the geometry of two (C-3 and C-6) of its three internal double bonds. The most common isomers are (3*E*,6*E*)-α-farnesene known as *trans*,*trans*-α-farnesene and (3*Z*,6*E*)-α-farnesene known as *cis*,*trans*-α-farnesene (**8-7**). (3*E*,6*E*)-α-Farnesene, which represents about 90% of the α-farnesene isomers in some apple and pear cultivars, accumulates in the surface wax of fruits during low-temperature storage. Conjugated trienes, such as (7*E*,9*E*)-2,6,10-trimethyldodeca-2,7,9,11-tetraen-6-ol, resulting from its oxidation, have been linked with the development of a serious physiological storage disorder known as superficial scald, which leds to injury of cell membranes. Scald manifests as brown or black patches on the fruit skin and in a bitter taste in the fruit. (3*Z*,6*E*)-α-Farnesene has been isolated from the perilla oil (*Perilla frutescens*, Lamiaceae) that dries faster than linseed oil; therefore it is used in the production of varnishes. The herb is used in Chinese medicine, either alone or in combination with other herbs, especially as a remedy for coughs and asthma. Both isomers act as insect pheromones. β-Farnesene can exist as two stereoisomers about the geometry of its central (C-6) double bond. The (6*E*)-isomer, known as *trans*-β-farnesene (**8-7**), is a constituent of various essentials oils, such as ginger oil. It is also released by aphids as an alarm pheromone upon death to warn away other aphids. Several plants have been shown to synthesise this pheromone as a natural insect repellent.



8-7, linear sesquiterpenic farnesenes

Cyclic sesquiterpenes (**8-8**) are found in large numbers in food volatiles and essential oils. The representatives of monocyclic hydrocarbons with six-membered cycle are α-bisabolene and β-bisabolene, α-zingiberene and *ar*-curcumene. Representatives of macrocyclic hydrocarbons are germacrene A, germacrene D and α-humulene. Bicyclic hydrocarbons with two six-membered rings are α-selinene, valencene, α-, β-, γ- and δ-cadinene. Muurolenes are isomeric with cadinenes,[1] while examples of other structures are β-caryophyllene and α-bergamotene. Some sesquiterpene hydrocarbons are precursors for the biosynthesis of other important compounds. For example, germacrene D is the precursor of two

[1]The name cadinene is sometimes used to refer to all sesquiterpenes having the structure of cadalane (4-isopropyl-1,6-dimethyldecahydronaphthalene). However, a large number of isomers exist with different positions and stereochemistry of the double bonds. These compounds are divided into four subgroups according to the stereochemistry of the isopropyl group at C-1 and hydrogen atoms on C-4a and C-8a. Cadinenes (1*S*,4a*R*,8a*R*), muurolenes (1*S*,4a*S*,8a*R*), amorphenes (1*S*,4a*R*,8a*S*) and bulgarenes (1*S*,4a*S*,8a*S*) have been identified.



8-8, cyclic sesquiterpenic hydrocarbons

major odour-active components of grapefruit peel, valencene and nootkatone. Valencene is the main volatile emitted by flowers of the vine (*Vitis vinifera*, Vitaceae) and a precursor of ketone nootkatone in grapefruits. (−)-δ-Cadinene is a precursor of the dimeric sesquiterpenoid pigment of cotton plants (*Gossypium hirsutum* and other species, Malvaceae) called gossypol.

β-Caryophyllene is a common component of many essential oils. For example, the essential oil of black pepper (see Table 8.32, later) contains about 20% of β-caryophyllene. Its α-isomer humulene (also known as α-caryophyllene) occurs in the hops essential oil (*Humulus lupulus*, Cannabaceae); both compounds are often present in the mixture. Common components of essential oils are also bisabolenes. Higher quantities of β-bisabolene (10–15%) and also α-zingiberene (22–30%), *ar*-curcumene (20%), α-selinene and β-farnesene are found in ginger essential oil. The essential oil of white sandalwood (*Santalum album*, Santalaceae) contains, in addition to sesquiterpenic alcohols as major components, many sesquiterpenic hydrocarbons, including α-bergamotene *ar*-curcumene, β-bisabolene and many others. Trivial and systematic names of the main sesquiterpenic hydrocarbons are listed in Table 8.3.

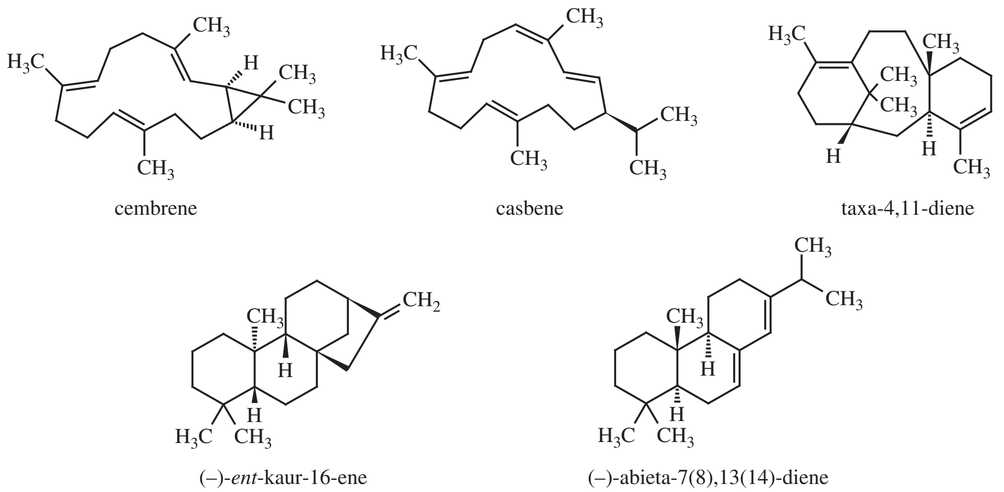
Diterpenes

Although not directly involved as odour-active food components, diterpenes are widespread in the plant kingdom, where they mainly occur as components of resins of conifers and juices of the aster (sunflower) family (Asteraceae) and spurge family (Euphorbiaceae) plants. Diterpenic hydrocarbons are precursors of numerous diterpenoids, many of which are biologically active substances. More than 3000 different diterpenoid structures have been defined, all of which appear to be derived from geranylgeranyl diphosphate. Like monoterpenes and sesquiterpenes, diterpenes are mostly cyclic compounds. Examples of diterpenoid hydrocarbons (**8-9**) are tricyclic hydrocarbon (−)-*ent*-kaur-16-ene, tetracyclic hydrocarbon (−)-abieta-7(8),13(14)-diene and macrocyclic compounds cembrene, casbene and taxa-4,11-diene.

All of these structures are central to a wide variety of natural products found both in plants and in animals. For example, cembrene is a pheromone of termites; casbene occurs in castor beans (*Ricinus communis*, Euphorbiaceae) as an antifungal substance and is a precursor of other biologically active diterpenoids, such as phorbol (see **8-25**, later) of the same plant family. Many species of the *Euphorbiaceae* family are regarded as toxic because their

Table 8.3 Trivial and systematic names of some sesquiterpenic hydrocarbons.

Trivial name	Systematic name (IUPAC)
(−)-(*E*)-α-Bergamotene	(1*S*,5*S*,6*R*)-2,6-Dimethyl-6-(4-methylpent-3-en-1-yl)bicyclo[3.1.1]hept-2-ene
(*E*)-α-Bisabolene	(*E*)-1-Methyl-4-(6-methylhepta-2,5-dien-2-yl)cyclohex-1-ene
(−)-β-Bisabolene	(*S*)-1-Methyl-4-(6-methylhepta-1,5-dien-2-yl)cyclohex-1-ene
(*Z*,*E*)-α-Farnesene	(3*Z*,6*E*)-3,7,11-Trimethyldodeca-1,3,6,10-tetraene
(*E*,*E*)-α-Farnesene	(3*E*,6*E*)-3,7,11-Trimethyldodeca-1,3,6,10-tetraene
(*E*)-β-Farnesene	(*E*)-7,11-Dimethyl-3-methylidenedodeca-1,6,10-triene
(+)-Germacrene A	(1*E*,5*E*,8*R*)-1,5-Dimethyl-8-prop-1-en-2-ylcyclodeca-1,5-diene
(−)-Germacrene D	(1*E*,6*E*,8*S*)-1-Methyl-5-methylidene-8-propan-2-ylcyclodeca-1,6-diene
α-Humulene	(1*E*,4*E*,8*E*)-2,6,6,9-Tetramethylcycloundeca-1,4,8-triene
(+)-α-Cadinene	(1*S*,4a*R*,8a*R*)-1-Propan-2-yl-4,7-trimethyl-1,2,4a,5,6,8a-hexahydronaphthalene
(+)-β-Cadinene	(1*S*,4a*R*,8a*R*)-4,7-Dimethyl-1-propan-2-yl-1,2,4a,5,8,8a-hexahydronaphthalene
(+)-γ-Cadinene	(1*S*,4a*R*,8a*R*)-7-methyl-4-methylidene-1-propan-2-yl-1,2,3,4a,5,6,8a-hexahydronaftalene
(+)-δ-Cadinene	(1*S*,8a*R*)-4,7-Dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene
(−)-β-Caryofyllene	(1*R*,4*E*,9*S*)-8-Methylen-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene
(+)-*ar*-Curcumene	(*S*)-1-(1,5-Dimethylhex-4-enyl)-4-methylbenzene
(+)-α-Muurolene	(1*S*,4a*S*,8a*R*)-4,7-Dimethyl-1-propan-2-yl-1,2,4a,5,6,8a-hexahydronaftalen
(+)-α-Selinene	(4a*R*,7*R*)-1,4a-Dimethyl-7-prop-1-en-2-yl-3,4,4a,5,6,7,8,8a-octahydronaphthalene
(+)-Valencene	(1*R*,7*R*,8a*S*)-1,8a-Dimethyl-7-prop-1-en-2-yl-1,2,3,5,6,7,8,8a-octahydronaphthalene
(−)-α-Zingiberene	(2*S*,5*R*)-2-Methyl-5-(6-methylhept-5-en-2-yl)cyclohexa-1,3-diene



8-9, cyclic diterpenic hydrocarbons

latex can cause poisoning in humans and animals, skin dermatitis, cell proliferation and tumour promotion. Most of the biological effects are due to the fatty acid esters of phorbol, such as 13-acetyl-12-myristoyl phorbol. The principal toxin of common yew (*Taxes baccarat*, Fabaceae) has been shown to be a mixture of at least 11 alkaloids based on the taxa-4,11-diene skeleton, *ent*-caurene is a precursor of plant hormones gibberellins, flavour-active diterpenoid alcohols cafestol and kahweol (see **8-25**, later) found in unroasted coffee beans, diterpenoid antioxidants, such as carnosic acid and bitter carnosol (see **10-181**) occurring in a medical herb rosemary (*Rosmarinus officinalis*, Lamiaceae) and sweet stevioside (see Section 11.3.2.1.3) in the leaves of South American stevia

(*Stevia rebaudiana*, Asteraceae), known as sweet leaf. Abietadiene is a precursor of abietic acid (see **3-156**) in resins in coniferous trees.

8.2.1.1.2 Other hydrocarbons

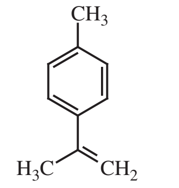
Aliphatic and alicyclic hydrocarbons

Saturated and unsaturated hydrocarbons with odd and even numbers of carbon atoms in the molecule (about C11–C35) are present as the primary substances in all vegetable oils and animal fats. Alkanes, alkenes, alkadienes and alkatrienes also arise as oxidation products of unsaturated fatty acids, catalysed by lipoxygenases or by autoxidation of fatty acids during food storage and processing. Only the lower hydrocarbons can play a role as odour-active substances. The main hydrocarbons resulting from oxidation of unsaturated fatty acids are ethane from linolenic acid, pentane and butane from linoleic acid and hexane and octane from oleic acid. The immediate precursors of hydrocarbons are the fatty acid hydroperoxides (Table 8.4). The unsaturated hydrocarbons are predominantly (*Z*)-isomers. Numerous other hydrocarbons, including alicyclic hydrocarbons, appear as secondary lipid oxidation products.

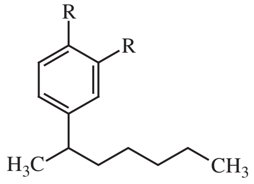
Some aliphatic, alicyclic and aromatic hydrocarbons may be products of oxidation and degradation of substances other than lipids; for example, penta-1,3-diene arises by decarboxylation of sorbic acid (see Section 11.2.1.1.2), which is used as a preservative.

Aromatic hydrocarbons

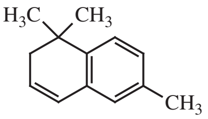
Aromatic hydrocarbons are relatively rare natural components of foods. An important natural component of essential oils of many spices and vegetables is *p*-cymene (1-isopropyl-4-methylbenzene, **8-3**). Together with the related hydrocarbon α,*p*-dimethylstyrene (**8-10**), *p*-cymene is also formed by degradation of citral. Various alkyl benzenes and alkyl xylenes (**8-10**) are found in small



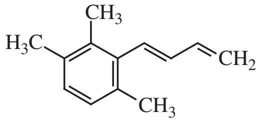
α,*p*-dimethylstyrene



olive oil alkylbenzenes (R = H)
olive oil alkylxylenes (R = CH_3)



1,2-dihydro-1,1,6-trimethylnaphthalene



(*E*)-1-(2,3,6-trimethylphenyl)-buta-1,3-diene

8-10, selected aromatic hydrocarbons

Table 8.4 Some hydrocarbons formed by oxidation of unsaturated fatty acids.

Hydrocarbon	Hydroperoxy acid	Fatty acid
Dec-1-ene	(*Z*)-8-Hydroperoxyoctadec-9-enoic	Oleic
Non-1-ene	(*E*)-9-Hydroperoxyoctadec-10-enoic	Oleic
Octane	(*E*)-10-Hydroperoxyoctadec-8-enoic	Oleic
Hexane	(*Z*)-11-Hydroperoxyoctadec-9-enoic	Oleic
(*Z*)-Deca-1,4-diene	(9*Z*,12*Z*)-8-Hydroperoxyoctadeca-9,12-dienoic	Linoleic
(*Z*)-Nona-1,3-diene	(10*E*,12*Z*)-9-Hydroperoxyoctadeca-10,12-dienoic	Linoleic
(*Z*)-Oct-2-ene	(8*E*,12*Z*)-10-Hydroperoxyoctadeca-8,12-dienoic	Linoleic
Hept-1-ene	(9*Z*,12*Z*)-11-Hydroperoxyoctadeca-9,12-dienoic	Linoleic
Hex-1-ene	(9*Z*,13*E*)-12-Hydroperoxyoctadeca-9,13-dienoic	Linoleic
Pentane	(9*Z*,11*E*)-13-Hydroperoxyoctadeca-9,11-dienoic	Linoleic
Butane	(9*Z*,12*Z*)-14-Hydroperoxyoctadeca-9,12-dienoic	Linoleic
(3*Z*,6*Z*)-Nona-1,3,6-triene	(10*E*,12*Z*,15*Z*)-9-Hydroperoxyoctadeca-10,12,15-trienoic	Linolenic
(2*Z*,5*Z*)-Octa-2,5-diene	(8*E*,12*Z*,15*Z*)-10-Hydroperoxyoctadeca-8,12,15-trienoic	Linolenic
(*Z*)-Hepta-1,4-diene	(9*Z*,12*Z*,15*Z*)-11-Hydroperoxyoctadeca-9,12,15-trienoic	Linolenic
(*Z*)-Hexa-1,3-diene	(9*Z*,13*E*,15*Z*)-12-Hydroperoxyoctadeca-9,13,15-trienoic	Linolenic
(*Z*)-Pent-2-ene	(9*Z*,11*E*,15*Z*)-13-Hydroperoxyoctadeca-9,11,15-trienoic	Linolenic
But-1-ene	(9*Z*,12*Z*,15*Z*)-14-Hydroperoxyoctadeca-9,12,15-trienoic	Linolenic
Ethane	(9*Z*,12*Z*,14*E*)-16-Hydroperoxyoctadeca-9,12,14-trienoic	Linolenic

amounts in olive oil. The degradation products of carotenoids are, for example, 1,2-dihydro-1,1,6-trimethylnaphthalene (**8-10**), which is an odorant of tomatoes (see Section 9.9.5.2.1) and (*E*)-1-(2,3,6-trimethylphenyl)buta-1,3-diene, present as a potent odorant in wines.

Undesirable contaminants include monocyclic aromatic hydrocarbons (MAHs), toluene, xylenes and ethyl benzene, which may be present in small quantities in foods as exogenous contaminants, along with polycyclic aromatic hydrocarbons (PAHs, see Section 12.2.5.1). Together with benzene and styrene (vinyl benzene), these hydrocarbons are also formed as processing contaminants (see Section 12.2.6).

8.2.1.2 Properties and reactions

Terpenic hydrocarbons are stable in the absence of air, but are easily oxidised in air, especially at higher temperatures. Their autoxidation proceeds by similar mechanisms as autoxidation of unsaturated fatty acids and depends greatly on the hydrocarbon structure. The primary autoxidation products are hydroperoxides. In branched hydrocarbons, the hydroperoxyl group mainly occurs in the secondary or tertiary carbon adjacent to the quaternary carbon of the double bond. The final autoxidation products are usually epoxides, alcohols and ketones. The primary site of attack in olefins is the carbon adjacent to the double bond, as in monounsaturated fatty acids. Hydroperoxides can also be formed by the addition of singlet oxygen to the olefin double bond.

An example is the oxidation of limonene, the (+)-(*R*)-isomer of which is the major component of essential oils from the peel of citrus fruits. The oxidation products are the alcohols (2*R*,4*S*)-carveol, known as (+)-(*E*)-carveol, and (1*S*,4*S*)-carveol, known as (+)-(*Z*)-carveol, ketone (+)-(*S*)-carvone (see **8-50**, later), which is an important component of caraway essential oil, and isomeric limonene-1,2-epoxides, also known as limonene oxides. The reaction mechanism, including the hydroperoxide intermediates, is shown in Figure 8.1. Analogous (+)-limonene products also result from oxidation of some microorganisms, which is used in their biosynthetic production. Grapefruit peel oil aroma, a strange, extraneous type of aroma normally not present in oranges, may arise as an off-flavour through metal-catalysed oxidation or photooxidation of the hydrocarbon (+)-valencene (**8-8**) to ketone (+)-nootkatone via hydroxylation to the corresponding alcohol (+)-β-nootkatol (see **8-23**, later) and its oxidation in orange juices. Cyclisation of β-ionone to 1,1,6-trimethyl-1,2-dihydronaphthalene (**8-10**) in fruits containing carotenoid pigments causes off-flavour in fruit juices and wines (see Section 9.9.5.2.1).

Autoxidation of saturated hydrocarbons is, like autoxidation of saturated fatty acids, important at higher temperatures (around 150 °C). The final odorous products formed are mainly fatty acids, their lactones, alcohols and ketones with fewer carbon atoms in the

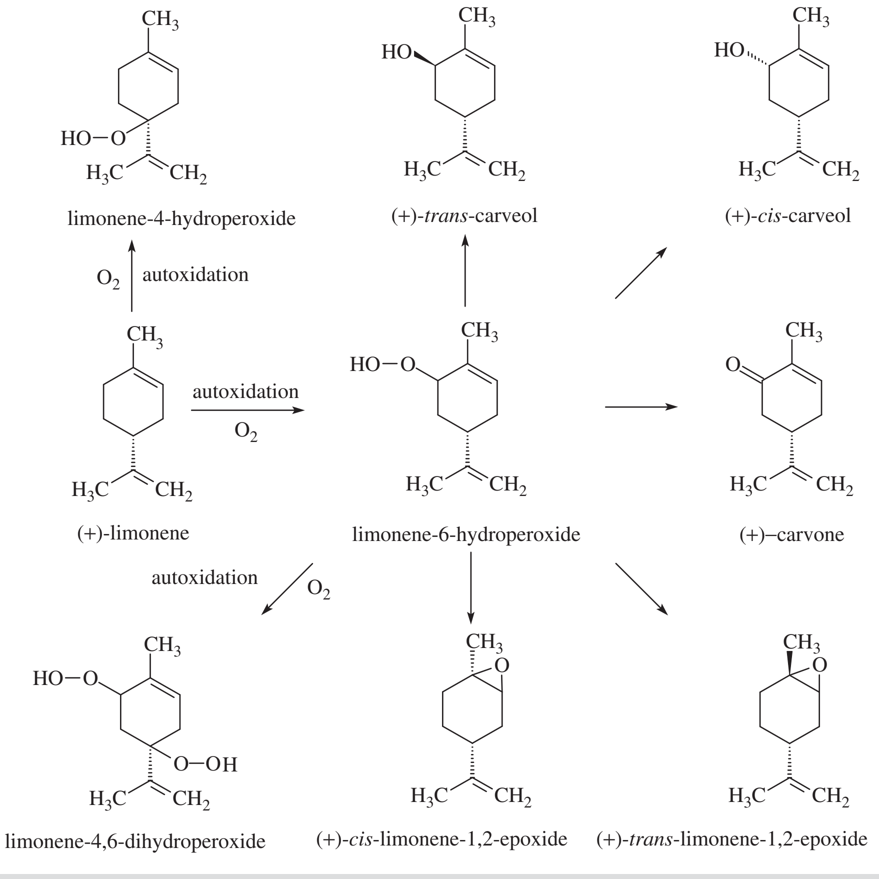


Figure 8.1 Main products of (+)-limonene oxidation.

molecule. The oxidation also occurs in aromatic and condensed aromatic hydrocarbons. The oxidation products formed chemically or *in vivo* are regarded as the carriers of the carcinogenic effects of these hydrocarbons (see Section 12.2.5).

8.2.2 Alcohols

Alcohols and phenols can be formally considered as the primary oxidation products of hydrocarbons. Alcohols are primary and secondary odour- and taste-active substances of plant and animal origin. Typical compounds are primary, secondary and tertiary aliphatic, alicyclic, aromatic and heterocyclic alcohols and alcohols containing more hydroxyl groups (diols, triols and higher polyols). Free primary alcohols and their esters mainly act as odour-active compounds, especially in fruits and alcoholic beverages. Lower aliphatic saturated and unsaturated alcohols are natural odour-active substances, especially monoterpenic and sesquiterpenic alcohols. Higher terpenoids can play roles as odour-active substances through their degradation products. Slight or no odour-activity is shown by higher aliphatic alcohols (such as the fatty alcohols, which are components of waxes), polar aliphatic and alicyclic diols, triols, other polyols, diterpenic, triterpenic alcohols and sterols, which accompany lipids, amino alcohols, such as 2-aminoethanol and choline, that are present as part of the phospholipids, hydroxycarboxylic acids and other polar hydroxy derivatives. Some substances, such as glycerol, sugar alcohols (exhibiting a sweet taste) and hydroxycarboxylic acids (sour taste), however, are important taste-active substances.

Alcohols (especially terpenic alcohols) that have less than 15–18 carbon atoms in the molecule are used for food flavouring. The use of higher alcohols is an exception. Lower alcohols are used for the production of esters, acetals and other flavour-active compounds or are used as food additives (e.g. as solvents).

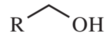
8.2.2.1 *Classification structure, terminology and occurrence*

8.2.2.1.1 *Aliphatic and alicyclic alcohols*

Methanol

Methanol (**8-11**) is a member of the homologous series of saturated aliphatic alcohols found in plants in the form of various esters, most often in pectins and esters of aromatic carboxylic acids (benzoic, salicylic, cinnamic and others). Free methanol results mainly by hydrolysis of pectin catalysed by plant pectin esterases: therefore, it occurs regularly in small amounts as a component of all natural fruit juices and is present in larger amounts in wines and fruit distillates.

The methanol content in citrus juices ranges from 24 to 47 mg/l, its content in ciders is 36–88 mg/l, and in blackcurrant juices reaches 70–176 mg/l. The methanol content of fruit wines is higher; usually ranges from 20 to 240 mg/l, but, exceptionally, it may reach even more than 600 mg/l, because a further portion of the pectin is hydrolysed during the fermentation process. The amount of methanol formed in wines depends on many factors. Red wines have about twice as high a methanol content as white wines. Vodka has



8-11, aliphatic alcohols

methanol, R = H
ethanol, R = CH_3
propan-1-ol, R = CH_2CH_3
butan-1-ol, R = $CH_2CH_2CH_3$
2-methylpropan-1-ol, R = CH_3CHCH_3
2-methylbutan-1-ol, R = $CH_3CHCH_2CH_3$
3-methylbutan-1-ol, R = $CH_2CH(CH_3)_2$
pentan-1-ol, R = $[CH_2]_3CH_3$
hexan-1-ol, R = $[CH_2]_4CH_3$
heptan-1-ol, R = $[CH_2]_5CH_3$

a very small amount of methanol (79–158 mg/l). Spirits produced by distilling wine (such as brandy or cognac) have methanol contents similar to those of wines (320–400 mg/l). Fruit brandies (distilled from fermented stone fruits) tend to have much higher methanol contents. The content of methanol in cherry brandy is usually 0.48–0.95% (4800–9500 mg/l) and in plum brandy around 1.2% (12 000 mg/l).

Ethanol

Ethanol is a natural metabolite and a small amount of ethyl alcohol (0.003–0.015% in blood) is necessarily present in our bodies, even if we do not drink alcohol. Ethanol (**8-11**) is generally not considered an important flavouring, but still has a significant impact on the flavour and energy value of many beverages. The energy value of ethanol is 29 kJ/g (7 kcal/g). Ethanol, bound in various esters, for example in essential oils, is a common volatile component of many foods (in small amounts). Free ethanol, together with carbon dioxide and many minority substances, is formed as the main product of anaerobic degradation of sugars by yeast, the so-called alcoholic fermentation or alcoholic glycolysis. Therefore, ethanol is present in variable amounts in alcoholic beverages and is also present in batter, bread and all fermented milk products. For example, yoghurt and other dairy products contain ethanol in quantities ranging from 0.04 to 0.05%. Alcoholic fermentation is a sequence of reactions, which generates pyruvic acid from sugars as the key intermediate (Figure 8.2). The decarboxylation of pyruvic acid by pyruvate decarboxylase yields acetaldehyde (ethanal), which is reduced to ethanol by alcohol dehydrogenase. The amount of ethanol depends on the amount of fermentable sugars in the raw material, type and strain of yeast used, the fermentation temperature, nutrient content in the medium and other factors. The same reaction also takes place in plants when there is a reduced oxygen level. For the industrial production of ethanol, certain strains of *Saccharomyces cerevisiae* yeast are employed, which are able to ferment glucose, mannose, fructose, saccharose, xylulose and raffinose. The ability to ferment galactose, maltose and melibiose is variable; trehalose, lactose and xylose are not fermented at all. Glucose, fructose and saccharose can enter yeast cells directly (after cleavage of saccharose by invertase); maltose requires specific permeases to enter the yeast cells.

To produce ethanol from starchy materials, such as cereal grains, the starch must first be converted into fermentable sugars, which are formed by starch degradation by amylases and maltases (see Section 4.5.6.1.3). For the production of beer, for example, yeast strains are selected that metabolise maltose and maltotriose (which

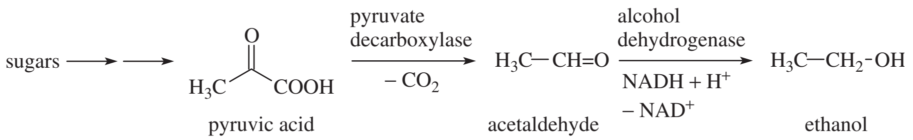


Figure 8.2 Formation of ethanol in alcoholic fermentation of sugars.

is fermented to varying degrees), the two sugars that prevail in the wort. According to the type of yeast used in the fermentation process, beers are differentiated into ales and lagers. Ales use yeast strains active at warmer temperatures and rise to the top of the wort (top-fermenting type of *S. cerevisiae*), while lagers use strains of yeast that are active at cooler temperatures and the yeast resides settle at the bottom of the tank (bottom-fermenting type). Some bacteria and fungi also produce ethanol from sugars. For example, the natural fermentative agent in anaerobic ethanol production from glucose is the gram-negative bacterium *Zymomonas mobilis*. Recent efforts have concentrated on natural renewable and relatively low cost lignocellulose waste that can be fermented to give ethanol by either bacteria (such as strains of the wood-decaying bacteria of the genus *Paenibacillus* that can break down and digest hemicelluloses containing xylose as the main sugar or thermophilic bacteria *Clostridium thermohydrosulfuricum* strains) or yeasts (such as *Candida utilis*, *C. tropicalis* and *Rhodotorula glacilis*). Starchy materials can similarly be fermented by mixed cultures of yeasts and molds (such as *Aspergillus niger* and *Saccharomyces cerevisiae*).

The ethanol content of beers varies over a relatively wide range. According to the original concentration of the extract in the wort before fermentation,[2] beers produced in the Czech Republic are classified as **light** if they have up to 7% m/m extract, **standard** (**table**) **beers** with an extract of 8–10% m/m, **lagers** with an extract of 11–12% m/m, **special beers** with a minimum extract of 13% m/m and **porters** with a minimum extract of 18% m/m. In the packaging and accompanying commercial documents, the original wort content is rounded down to the nearest whole number. For example, beer with an extract of 10.2% m/m is regarded as standard beer.

Instead of the original extract declaration, the packaging often declares ethanol content in per cent by volume (alcoholic strength or alcohol by volume, in short ABV), usually recognised as a long-term average value, for example 5.0%. Designation according to the ethanol content is also mandatory for beers with reduced alcohol content (from 0.5 to 1.2% by volume) and **non-alcoholic beers**, which contain up to 0.5% alcohol by volume. Similar classification of beers is also used in other EU countries. For example, the Pilsner type of beer known as Pilsner Urquell, produced by bottom-fermentation technology in the Czech Republic, has an original wort concentration of 11–12% and ethanol content of about 3.6% w/w. Beers for diabetics are low carbohydrate beers with higher alcohol content. Dark beers have an original wort content that is generally higher (13–20% w/w). The dark beers Stout, Porter and Guinness, produced in the United Kingdom, are usually bottom fermented and have high ethanol contents (up to 6.8% w/w).

Ethanol content in wines varies from 8–9% to 18–18.5% by volume, depending on the content of sugars in the must (see Section 4.2.2.6). Normal table wines contain 10–14% ethanol by volume, while dessert wines of the sherry type contain 17–24% ethanol by volume. The ethanol content in grape brandy, spirits and other spirits is around 40% by volume, and a number of special products have lower alcohol content, but some spirits have also higher alcohol content. In the United States, the proof of an alcoholic beverage is twice its alcohol content expressed as percentage by volume at 60 °F (15.6 °C). So an 80-proof whiskey (whisky) is 40% ethanol.

Milk contains a small amount of ethanol, about 0.003% on average, yoghurts and other dairy products obtained by fermentation contain ethanol at levels not exceeding 0.04–0.05%. Of the dairy products, only fermented mare's milk (called kumiss or kumys) has higher alcohol content (1–3%).

Higher alcohols

In addition to ethanol, alcoholic fermentation produces a series of higher aliphatic alcohols (**8-11**) with a strong aroma, which are collectively known as the **fusel oil alcohols**. These alcohols accompany ethanol in beer, wine and other spirits, but also in dough and fermented dairy products (Table 8.5). Fusel oil alcohols are produced either by catabolic processes (some aminocarboxylic acids are their precursors) or anabolic processes (they are formed from sugars in the synthesis of aminocarboxylic acids). The immediate precursors of fusel oil alcohols are aldehydes generated as byproducts of metabolism. Alcohol dehydrogenases reduce these aldehydes to the corresponding alcohols. Formation of fusel oil alcohols is described in Section 2.5.1.3.2, which deals with transamination and oxidative deamination of aminocarboxylic acids. The level of fusel oil alcohols in alcoholic beverages depends on the raw materials processed. In wines, for example, important variables are the grape variety (red wines contain somewhat higher amounts of fusel oil alcohols than white wines), conditions during fermentation and yeast strains used.

[2] A slurry of germinated barley seeds (barley malt) and brewing water (called mash) is heated at a temperature around 60 °C to allow the malt enzymes, mainly amylases but also proteases, to degrade starch and proteins. In general, a few hundreds of grams of are used for 1 l of beer. The process is stopped by heating and the solution obtained by filtration is the wort. Using the Balling's formula, the original wort extract p (% m/m) can be calculated from the ethanol content A (% m/m) and real extract E (% m/m; determined from the beer density after evaporation of ethanol and made up to original weight with water):

$$p = \frac{100\,(E + 2.0665A)}{100 + 1.0665A}$$

Table 8.5 Fusel oil and other higher alcohols in some alcoholic beverages (mg/l).

Alcohol	Beer	Wine	Whisky	Alcohol	Beer	Wine	Whisky
Fusel oil alcohols				2-Phenylethanol	4–102	5–138	0.6–131
Propan-1-ol	4–60	11–93	20–187	Tyrosol	0.6–29	5–45	-
Butan-1-ol	-	3–9	-	Tryptophol	0.2–12	0–1.6	-
2-Methylpropan-1-ol	2–98	15–184	110–670	***Other alcohols***			
(*S*)-2-Methylbutan-1-ol	3–41	12–311	60–1390	Butan-2,3-diol[a]	40–250	165–1615	-
3-Methylbutan-1-ol	19–160	40–523	150–1465	Glycerol	1100–3 200	1400–26 700	-

[a]Butan-2,3-diol is a mixture of isomers of which (2*R*,3*R*)-butan-2,3-diol, also known as (−)-D-butan-2,3-diol, prevails.

2-Methylpropan-1-ol, known as isobutyl alcohol or isobutanol (resulting from valine via 2-oxoisovalerate) and 3-methyl-1-ol, known as isoamyl alcohol (created from leucine via 2-oxoisocaproate) are the main alcohols found in fusel oils in relatively large amounts. Both alcohols have considerable influence on the aroma of alcoholic beverages. Fusel oils contain some other alcohols in smaller quantities, such as (−)-(*S*)-2-methyl-1-ol known as optically active amyl alcohol (produced from isoleucine via 2-oxo-3-methylvaleric acid), propane-1-ol (which is produced from threonine via 2-oxobutyric acid) and butane-1-ol (created as a byproduct of isoleucine biosynthesis from threonine, by decarboxylation of 2-oxovaleric acid and reduction of butanal that is formed as the decarboxylation product, Figure 8.3). In the butanol fermentation method employed in industrial

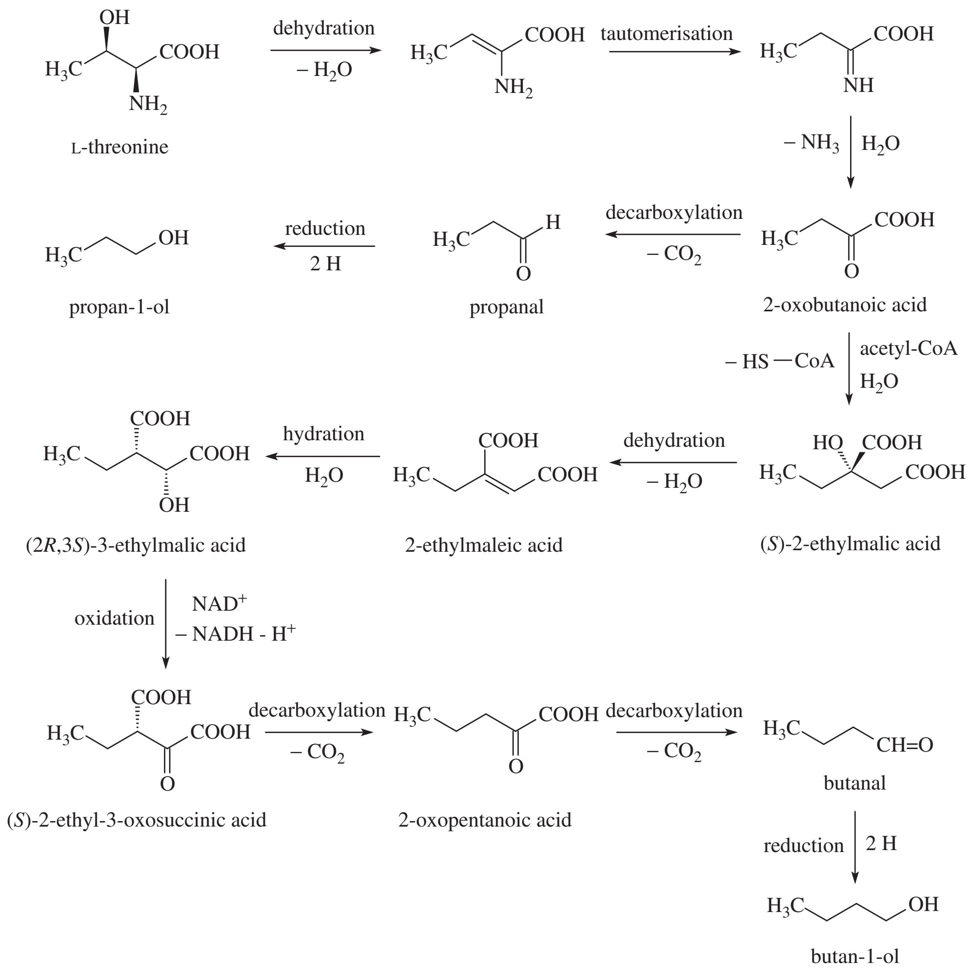
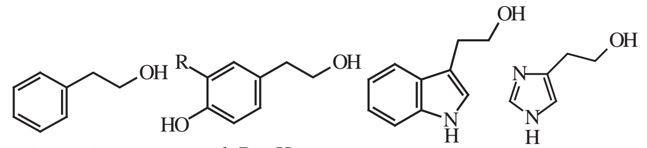


Figure 8.3 Formation of propan-1-ol and butan-1-ol from threonine.

processes (also known as acetone–butanol fermentation) with bacteria of the genus *Amylobacter*, butan-1-ol is the main product, followed by acetone, propan-2-ol (isopropyl alcohol) and ethanol. During fermentation processes, the aromatic aminocarboxylic acid phenylalanine forms 2-phenylethanol, also known as 2-phenylethyl alcohol (**8-12**), tyrosine yields non-volatile alcohol tyrosol (**8-12**), 3,4-dihydroxyphenylalanine (DOPA) yields 3-hydroxytyrosol (**8-12**) and tryptophol (**8-12**) is produced from tryptophan. Some mycobacteria, such as *Mycobacterium diernhoferi*, *M. fortuitum* and *M. chelonei* and some yeast strains (*Saccharomyces rouxii*) are known to oxidise histamine to give histaminol (**8-12**). Histaminol has also been found in red and white wines at concentrations of 0.3–1.1 mg/l.

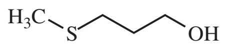


2-phenylethanol; tyrosol, R = H; hydroxytyrosol, R = OH; tryptophol; histaminol

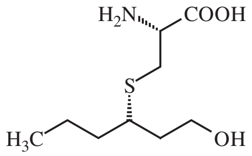
8-12, aromatic and heterocyclic alcohols derived from amino acids

Sulfur aminocarboxylic acids also yield some sulfur-containing aromatic and heterocyclic alcohols. Methional is formed from methionine and reduced to the corresponding alcohol methionol (**8-13**) in beers and wines. Important sulfur-containing alcohols formed in wines, including 3-mercapto-3-methyl-1-ol, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexan-1-ol, are formed as degradation products of cysteine conjugates (**8-14**) with sulfur-containing alcohols under the action of CS lyases. These sulfur-containing alcohols are characteristic components of wine aroma. For example, (+)-(*S*)-3-mercaptohexan-1-ol (**8-15**) has (on dilution) an interesting tropical fruit aroma, reminiscent of passion fruit (*Passiflora edulis*, Passifloraceae). The (−)-(*R*)-isomer has a fruitier aroma, reminiscent of grapefruit.

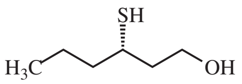
Some higher alcohols present in alcoholic beverages at low concentrations are even present in certain amounts in the raw materials. For example, pentan-1-ol, hexan-1-ol, heptan-1-ol (8-11), octan-1-ol and other higher alcohols are present in grape musts. They are formed by oxidation of essential fatty acids by lipoxygenases and by cleavage of fatty acid hydroperoxides by lyases, which are followed by reduction of the saturated or unsaturated aldehydes through the action of alcohol dehydrogenases (Figure 8.4) from the raw material and with the action of yeast alcohol dehydrogenases during fermentation. Detailed mechanisms of these reactions are given in Section 3.8.1.8.5.



8-13, methionol



8-14, 3-mercaptohexan-1-ol cysteine conjugate



8-15, (*S*)-3-mercaptohexan-1-ol

For example, reduction of propanal yields propan-1-ol, butan-1-ol is produced from but-2-enal or butanal, pentanal gives rise to pentan-1-ol, which is present in wines at levels of about 0.1 mg/l. Hexan-1-ol (the amount in wines is usually 0.3–12 mg/l) arises mainly by reduction of hexanal (Figure 8.5) and (*E*)-hex-2-enal (Figure 8.6) by alcohol dehydrogenases. It has a faint green and tallowy odour (see below). Hexanal forms by decomposition of linoleic acid (13*S*)-hydroperoxide with hydroperoxide lyase, (*E*)-hex-2-enal is formed by enzymatic cleavage of linolenic acid (13*S*)-hydroperoxide and isomerisation of the thus formed (*Z*)-hex-3-enal catalysed by enal isomerase. Reduction of methylketones produced by oxidation of fatty acids generates alkan-2-ols.

Alcohol dehydrogenases are quite specific to the substrate and reduce higher aldehydes (>C_5) very slowly. For this reason, the content of higher (fatty) alcohols in alcoholic beverages, as well as in fruits and vegetables, is relatively low and the main products of fatty acid oxidation are aldehydes and not alcohols.

In addition to aminocarboxylic and fatty acids, the precursors of higher alcohols may likewise become other food components. For example, minor degradation products of β-carotene, in addition to other compounds, are butan-1-ol, 2-methylpropan-1-ol and pentan-1-ol.

Unsaturated alcohols

The simplest aliphatic unsaturated alcohol, allyl alcohol (prop-2-en-1-ol), is formed from alliin during high temperature processing of garlic. Allyl alcohol was found to be one of the major degradation

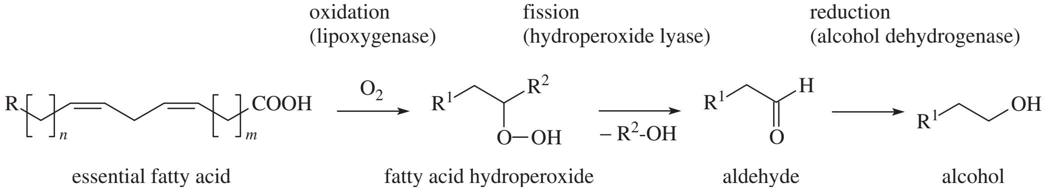


Figure 8.4 Formation of alcohols from fatty acids.

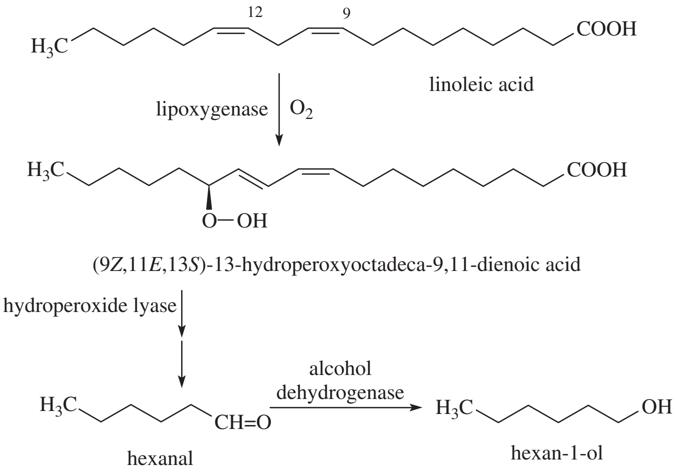


Figure 8.5 Formation of hexan-1-ol from linoleic acid.

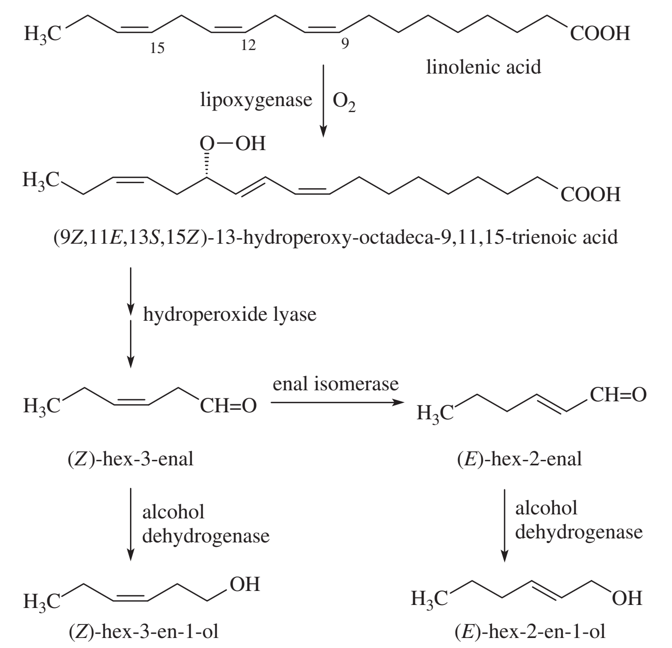


Figure 8.6 Formation of hex-2-en-1-ol and hex-3-en-1-ol from linolenic acid.

products of alliin heated at 80–200 °C in the presence of variable amounts of water. Its amount increased with the amount of added water and was the highest at 140 °C (37 mg/g amino acid). Allyl alcohol at a level of 1.1 mg/kg was also found in Boursin Ail cheese flavoured with garlic. It is proposed that allyl alcohol is formed either through [2,3]-sigmatropic rearrangement of alliin to the corresponding sulfenate, which decomposes to allyl alcohol and cysteine (Figure 8.7) or by transformation of alliin via thiosulfonium ion (see Section 8.2.9.1.4). Cysteine then decomposes further into acetaldehyde, hydrogen sulfide and ammonia.

Some unsaturated aliphatic alcohols arising from essential fatty acids are important flavour-active components of fresh fruits, vegetables and mushrooms. Hydroperoxides of fatty acids resulting

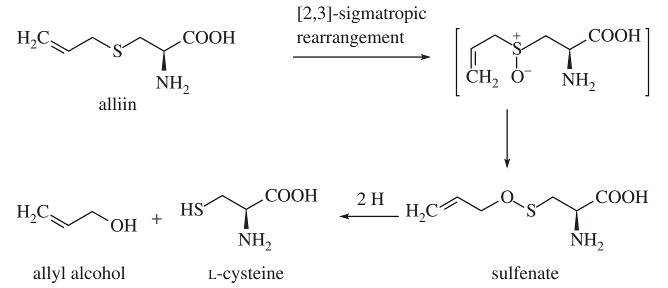
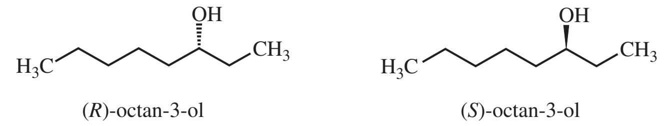


Figure 8.7 Formation of allyl alcohol from alliin.

from regioselective and stereospecific oxidation by lipoxygenases are broken down in different ways. In animal tissues, hydroperoxides are reduced to non-volatile hydroxycarboxylic acids by the enzyme glutathione peroxidase. In plants and fungi, hydroperoxides decompose by lyases, dehydrases, epoxygenases and hydroperoxide hydrolases. Differences in sensory quality of active products are related to the substrate and reaction specificity of these enzymes. The main products formed in plants are aldehydes, while mushrooms produce allyl alcohols.

Cleavage of 13- and 9-hydroperoxides of linoleic acid and, in particular, α-linolenic acid, which is the main fatty acid of green plants, by hydroperoxide lyases provides a range of aldehydes, significantly contributing to the fresh, herbal odour, called green odour, of fruits and vegetables (apples, plums, olives, leafy vegetables, tomatoes, peppers and other products). Decomposition of α-linolenic acid (13*S*)-hydroperoxide by hydroperoxide lyase yields (*Z*)-hex-3-enal that has pure green odour (Figure 8.6). Cleavage of (9*S*)-hydroperoxide of the same fatty acid provides (3*Z*,6*Z*)-nona-3,6-dienal with an oily and green odour (Table 8.6). (*Z*)-Alkenals can be subsequently transformed into (*E*)-alkenals by enal isomerases and reduced to (*Z*)- or (*E*)-alkanols by alcohol dehydrogenases (Figure 8.6). These products often have a characteristic smell of fruits and vegetables. For example, isomerisation of (*Z*)-hex-3-enal yields (*E*)-hex-2-enal, known as leaf aldehyde, on reduction this gives (*Z*)-hex-3-en-1-ol, which is trivially called leaf alcohol. Isomerisation of (3*Z*,6*Z*)-nona-3,6-dienal provides (2*E*,6*Z*)-nona-2,6-dienal with a typical aroma of fresh cucumbers. Hexanal (Figure 8.5) and some other aldehydes (Table 8.6) also contribute to the green odour.

The aroma of fresh mushrooms contains (−)-(*R*)-oct-1-en-3-ol, which arises in a similar way from linoleic acid (Figure 8.8). The formation of (10*S*)-hydroperoxide is catalysed by a specific lipoxygenase. Oct-1-en-3-ol is accompanied by oct-1-en-3-one, octan-1-ol and (*R*)-octan-3-ol (**8-16**). Other compounds also have the mushroom-like smell, such as (3*R*,5*Z*)-octa-1,5-dien-3-ol (**8-17**), which is produced analogously by decomposition of linolenic acid (10*S*)-hydroperoxide.

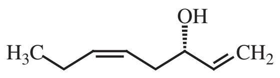


8-16, octan-3-ol isomers

Table 8.6 Trivial and systematic names of monoterpenic alcohols.[a]

Trivial name	Systematic name (IUPAC)
(+)-Borneol	(1*R*,2*S*,4*R*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol
(+)-Citronellol	(*R*)-3,7-Dimethyloct-6-en-1-ol
(+)-Fenchol	(*R*)-1,5,5-Trimethylbicyclo[2.2.1]heptan-6-ol
Geraniol	(*E*)-3,7-Dimethylocta-2,6-dien-1-ol
(−)-(*E*)-Hotrienol	(3*R*,5*E*)-3,7-Dimethylocta-1,5,7-trien-3-ol
(+)-Isomenthol	(1*S*,2*R*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-ol
(+)-(*E*)-Carveol	(1*R*,5*S*)-2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol
(−)-(*E*)-Carveol	(1*S*,5*R*)-2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol
(−)-Lavandulol	(*R*)-5-Methyl-2-prop-1-en-2-ylhex-4-en-1-ol
(+)-Linalool	(*S*)-3,7-Dimethylocta-1,6-dien-3-ol
(−)-Menthol	(1*R*,2*S*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-ol
(−)-2-Methylisoborneol	(1*R*-exo)-1,2,7,7-Tetramethylbicyclo[2.2.1]heptan-2-ol
(+)-Neomenthol	(1*S*,2*S*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-ol
Nerol	(*Z*)-3,7-Dimethylocta-2,6-dien-1-ol
(−)-Perillyl alcohol	[(*S*)-4-Prop-1-en-2-ylcyclohex-1-en-1-yl]methanol
(+)-(Z)-Sabinene hydrate	(1β,2β,5α)-4-Methyl-1-propan-2-ylbicyclo[3.1.0]hexan-4-ol
(+)-Terpinen-4-ol	(*S*)-4-Methyl-1-propan-2-ylcyclohex-3-en-1-ol
(+)-α-Terpineol	(*R*)-2-(4-Methyl-1-cyclohex-3-en-1-yl)propan-2-ol

[a]Numbers in the trivial names derived from *p*-menthane (4-isopropyl-1-methylcyclohexane) differ from the numbers of the names recommended by IUPAC. For example, (+)-(*E*)-dihydrocarvone is (1*S*,4*S*)-dihydrocarvone or (2*S*,5*S*)-2-methyl-5-prop-1-en-2-ylcyclohexanone; (+)-(*E*)-carveol is (2*R*,4*S*)-carveol or (1*R*,5*S*)-2-methyl-5-prop-1-en-2-ylcyclohex-2-en-1-ol.



8-17, (3*R*,5*Z*)-octa-1,5-dien-3-ol

In meat, oct-1-en-3-ol and oct-1-en-3-one are produced analogously by decomposition of 12-hydroperoxy-5,8,10,14-eicosatetraenoic acid, which is one of the arachidonic acid autoxidation products. When this decomposition occurs, it may cause an off-flavour.

Terpenic alcohols

Monoterpenic alcohols Monoterpenic alcohols, together with terpenic hydrocarbons and other terpenoids, are widespread components characteristic of various essential oils from flowers and other plant parts that are often used in perfumery. Many monoterpenic alcohols are also odour-active components of spices, fruits and vegetables. They exhibit frequently a sweet, heavy floral odour in different shades.

The most important monoterpenic acyclic alcohol with one double bond is citronellol. Alcohols with two double bonds include linalool, geraniol and nerol, and an example of an alcohol with three double bonds is hotrienol (**8-18**). (+)-Citronellol (β-citronellol) is a major component of essential oils of *Zieria citriodora* (syn. *Boronia citriodora*, Rutaceae, about 80%), a plant native to Australia and known as lemon-scented zieria. It also occurs in *Corymbia citriodora* (syn. *Eucalyptus citriodora*, Myrtaceae; 15–20%), an Australian tree known as lemon-scented gum or lemon eucalyptus. (−)-Citronellol isolated from natural sources is often called rhodinol. It is the predominant enantiomer in geranium (*Pelargonium graveolens* and other species, Geraniaceae) and Bulgarian Damask rose essential oils (*Rosa damascena* var. *bulgaria*, Rosaceae), which contain up to 50% citronellol. A mixture of both enantiomers is present in many essential oils, for example in the essential oil of aromatic citronella grass *Cymbopogon nardus* and *C. winterianus* (Poaceae) native to tropical Asia. (+)-Linalool (coriandrol) is found at a

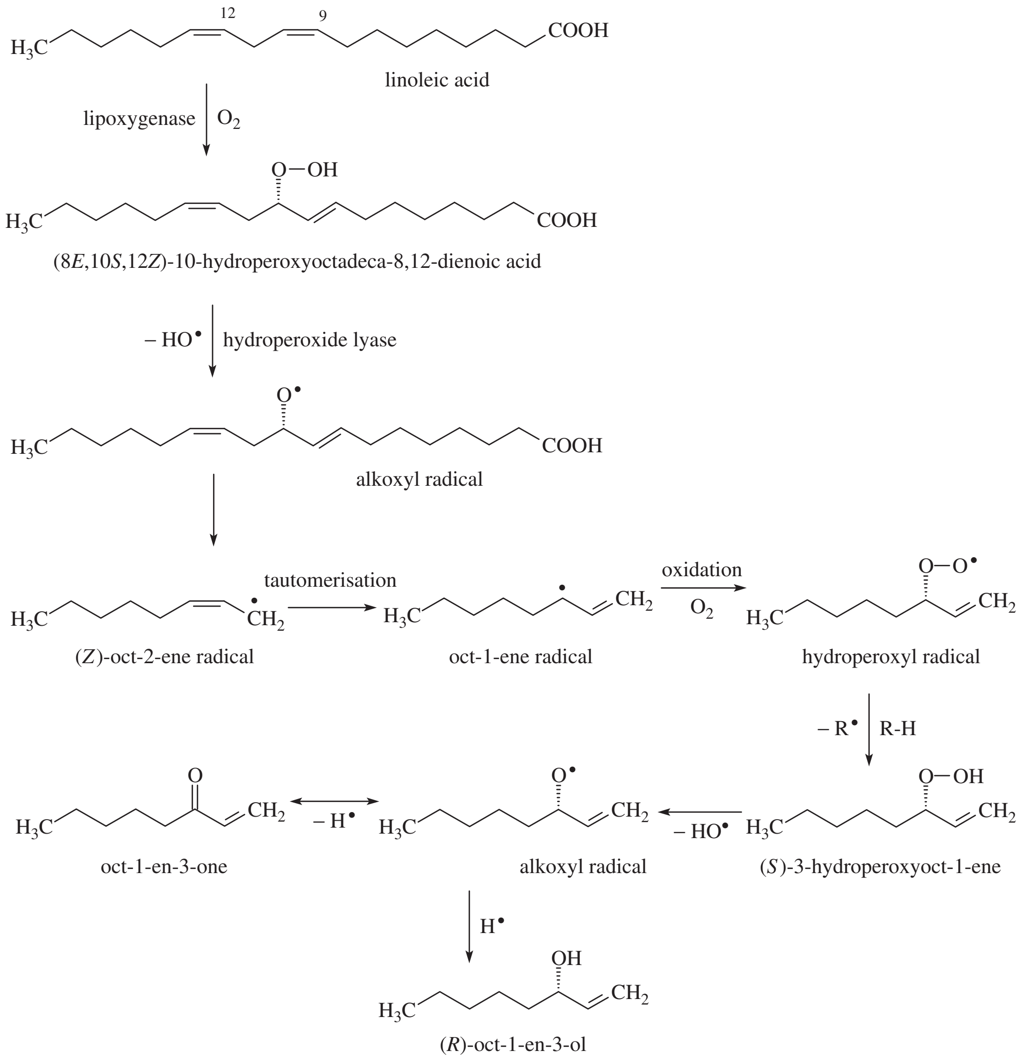
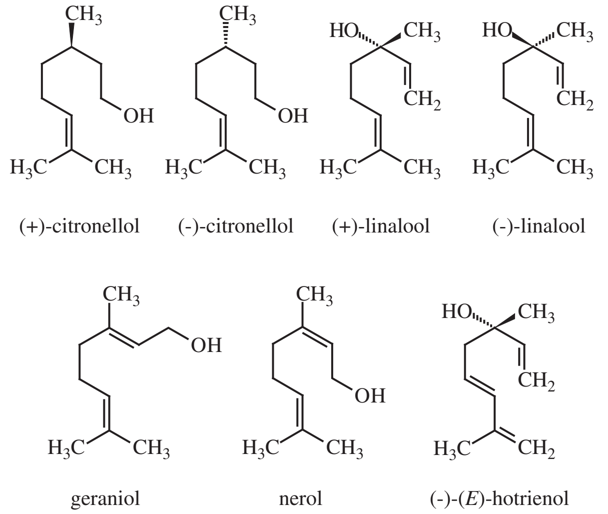


Figure 8.8 Formation of oct-1-en-3-ol and oct-1-en-3-one from linoleic acid.

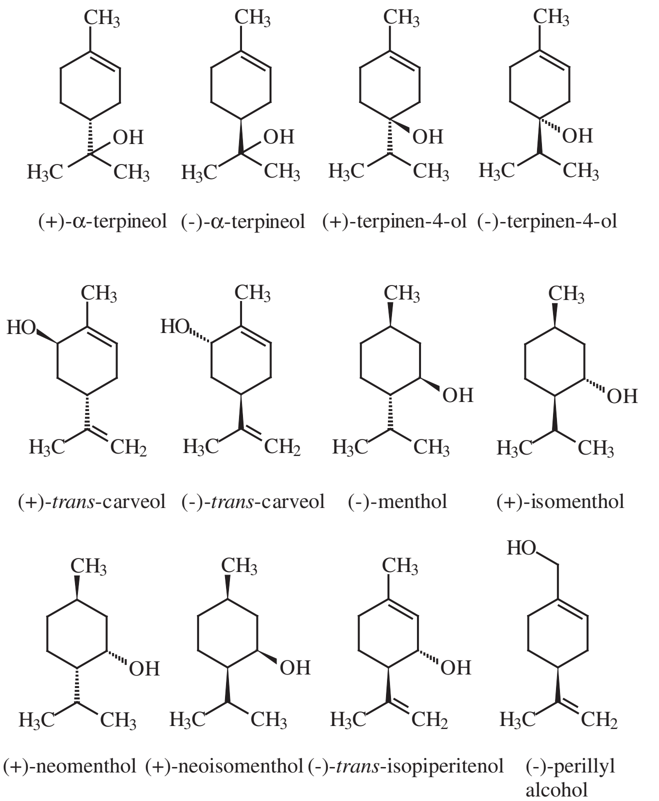


8-18, acyclic monoterpenic alcohols

level of 60–80% in coriander essential oil (see Table 8.32, later), (−)-linalool (licareol) is the major component (80–85%) of cinnamon essential oil. Geraniol is, at about 8%, a component of geranium oil, its *cis*-isomer, known as nerol, occurs in Bulgarian rose oil together with citronellol. Hotrienol is the product of linalool oxidation and further transformation of linalool oxidation products, as well as linalool oxides, lilac aldehyde and lilac alcohol. Hotrienol occurs in many essential oils, for example it is an odorous component of some aromatic wines and of the essential oil of elderberry flowers (*Sambucus nigra*, Adoxaceae).

Other common components of essential oils are the monoterpenic monocyclic alcohols α-terpineol (found in lilac, marjoram, cardamom, star anise oil and other oils), terpinen-4-ol (4-terpineol), also known as 4-carvomenthenol, is a component of the essential oils of pine (*Pinus* spp., Pinaceae), eucalyptus (*Eucalyptus* spp. Myrtaceae), marjoram and thyme (see Table 8.32, later). It is also the main component of the antiseptic essential oil of the Australian tree *Melaleuca alternifolia* (Myrtaceae), known as tea tree oil. It often occurs as a racemate. (+)-Terpinen-4-ol occurs at about

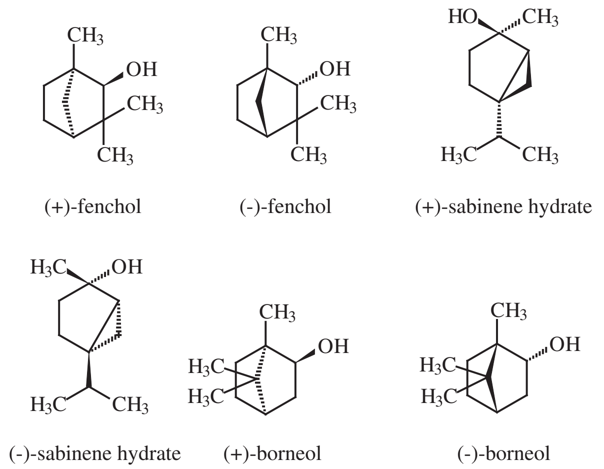
10% in the essential oil of lavender (*Lavandula* spp., Lamiaceae), (−)-terpinen-4-ol is a component of orange essential oil, (+)-(*E*)-carveol is an intermediate in the biosynthesis of the characteristic caraway oil component (see Table 8.32, later) (+)-carvone (see **8-50**, later) from (+)-limonene, while its isomer (−)-(*E*)-carveol (**8-19**) is an intermediate of biosynthesis of (−)-carvone from (−)-limonene. (−)-Carvone (see **8-50**, later) is an important component of spearmint essential oil (see Table 8.32, later).



8-19, monocyclic monoterpenic alcohols

(−)-Menthol, which evokes coolness through stimulation of the somatosensory system, is, along with its isomers (+)-isomenthol, (+)-neomenthol and (+)-neoisomenthol (**8-19**), a key component of peppermint essential oil (see Table 8.32, later). The starting compound for the biosynthesis of these alcohols is (−)-limonene, while one of the menthol precursors is (−)-(*E*)-isopiperitenol. Good-quality peppermint essential oil has a high menthol content, moderately high content of menthone and low content of (+)-(*R*)-pulegone and (+)-(*R*)-menthofuran. The (−)-(*S*)-enantiomers are found rarely in essential oils. Pulegone and menthofuran are undesirable because of their hepatotoxicity, and therefore appeared in the list of substances under Regulation (EC) No. 1334/2008, which should not be added to foods as flavouring. Their natural amount in foods is restricted to 2000 and 3000 mg/kg in micro breath freshening confectionery, to 250 and 500 mg/kg in other peppermint-containing confectionery, to 350 and 1000 mg/kg in chewing gums and to 100 and 200 mg/kg in peppermint-containing alcoholic beverages, respectively. The level of pulegone is restricted to 20 mg/kg in peppermint-containing non-alcoholic beverages.

An important precursor of (−)-perrillyl aldehyde (perillal) is (−)-perillyl alcohol. Fenchol (also known as α-fenchyl alcohol or fenchan-2-ol, **8-20**) is a bicyclic monoterpenic alcohol occurring as a minor component in citrus, fennel and sage essential oils. Another common compound is the bicyclic alcohol thujan-4-ol (sabinene hydrate), which occurs at a high level in the marjoram essential oil (see Table 8.32, later). Borneol (bornan-2-ol) is a component of camphor oils (*Cinnamomum camphora*, Lauraceae). Trivial and systematic names of selected monoterpenic alcohols are listed in Table 8.6.



8-20, bicyclic monoterpenic alcohols

Monoterpenic alcohols are found in the flowers of plants, fruits and other plant materials mainly as sensorially inactive glycosides. The predominant glycosides are β-D-glucopyranosides substituted with L-rhamnose, L-arabinose, D-apiose and acylated with malonic acid. Examples of these glycosides are α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosides, α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosides (β-rutinosides) and β-D-apiofuranosyl-(1→6)-β-D-glucopyranosides; less common glycosides are unsubstituted β-D-glucopyranosides. α-L-Arabinofuranosyl-(1→6)-β-D-glucopyranosides and β-D-apiofuranosyl-(1→6)-β-D-glucopyranosides of geraniol, β-D-glucopyranosides of geraniol, nerol, citronellol and β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (β-sophoroside) of citronellol occur, for example, as precursors of the corresponding alcohols in Bulgarian Damask rose flowers. In apricots and grapes, glycosides are mainly localised in the skin. Linalool, nerol and α-terpineol are present as β-D-glucosides, geraniol occurs in the form of β-rutinoside and linalool and α-terpineol as 6-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranosides. Glycosides of diols and other polyols have also been identified.

Complex enzymatic and non-enzymatic transformations of glycosides of monoterpenic alcohols and free alcohols during the development of flowers, fruit ripening or fermentation provide a range of new oxygenated monoterpenoids, which are often characteristic constituents of flowers, fruits and alcoholic beverages (wine and spirits). For example, a glycoside of terpenic alcohol can be enzymatically oxidised, or the free alcohol formed by hydrolysis can be oxidised, which is then transformed into other products.

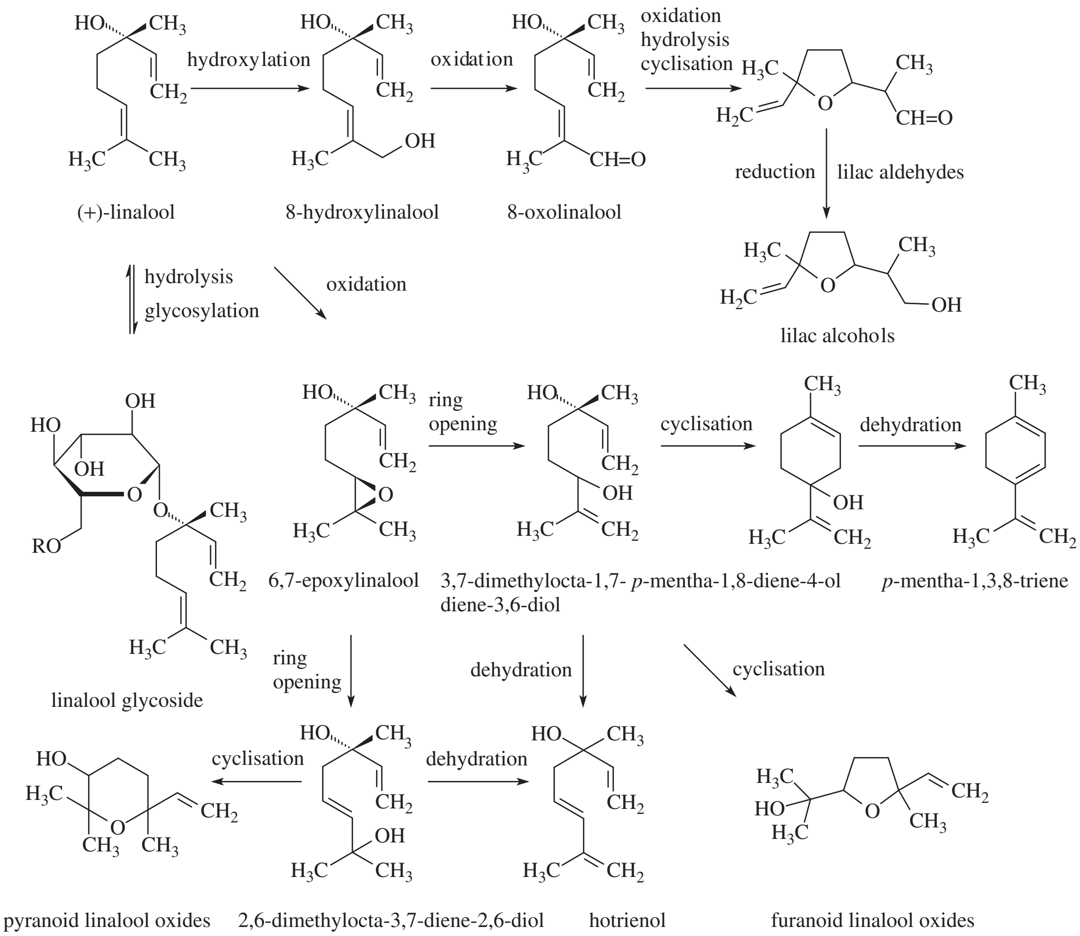
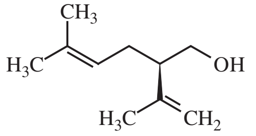


Figure 8.9 Reactions of linalool and formation of lilac alcohol, linalool oxides and hotrienol.

These transformations are shown in Figure 8.9 for the example of (+)-linalool and its glucoside. Linalool in lilac flowers (*Syringa vulgaris*, Oleaceae) is oxidised to 8-hydroxylinalool, which is the precursor of four (5'*S*)-isomers of lilac aldehydes, reduction of which yields lilac alcohols that are typical components of the odour of lilac flowers and honey from citrus flowers. Linalool glucoside can be oxidised analogously to 8-hydroxylinalool glucoside, hydrolysis of which provides free 8-hydroxylinalool. Under the acidic conditions in musts, especially during heating, glycosides are easily hydrolysed non-enzymatically to monoterpenic alcohols or their oxidation products, such as 6,7-epoxylinalool. Enzymatic ring opening of this epoxide yields either 3,7-dimethyl-1,7-octadien-3,6-diol or 2,6-dimethyl-3,7-octadien-2,6-diol. The former compound then becomes the precursor (after cyclisation and dehydration) of *p*-mentha-1,3,8-triene, which is the main flavour-active component of parsley essential oil. Its dehydration yields (−)-(*E*)-hotrienol, which is a component of many fruit aromas. In acidic solutions, spontaneous cyclisation of the mentioned diols yields furanoid and pyranoid linalool oxides. Linalool oxides are also present as glycosides.

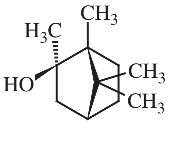
Illustrative of monoterpenic alcohols biosynthesised from two molecules of dimethylallyl diphosphate linked together head to head is (−)-lavandulol (**8-21**). Along with its acetate, lavandulol occurs



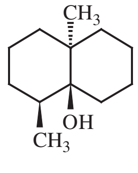
8-21, lavandulol

in the essential oil of common lavender (*Lavandula angustifolia*, syn. *L. officinalis*, Lamiaceae).

The characteristic odour of raw, wet land is caused by the presence of two terpenoids, monoterpenic alcohol (−)-2-methylisoborneol (**8-22**) and the sesquiterpenoid (−)-geosmin (**8-23**) produced by streptomycetes, myxobacteria and cyanobacteria. Both compounds cause an unpleasant odour in drinking water and some foods, such as canned mushrooms, wheat, coffee and meat of freshwater fish



8-22, 2-methylisoborneol



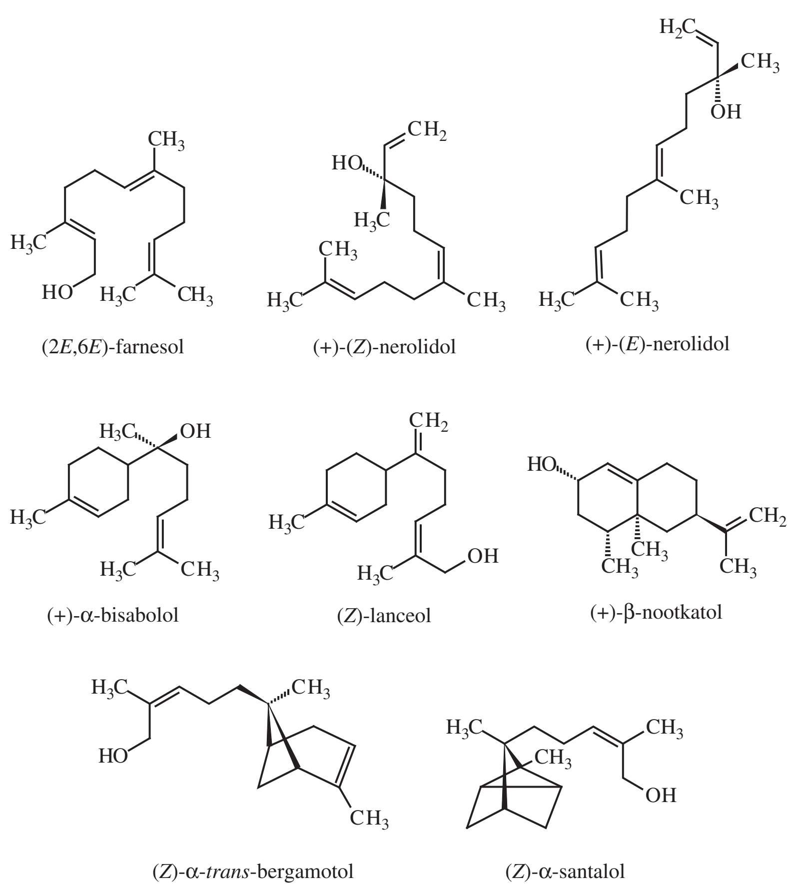
8-23, geosmin

(such as carp) that depend on the nutrients from benthos, animal (zoobenthos) and vegetable (phytobenthos) organisms inhabiting the bottom waters. 2-Methylisoborneol has been reported to occur in soil in quantities of about 4 μg/kg, which is more than 100-fold above its threshold value. Its reported occurrence in the green coffee beans (80–420 ng/kg in Arabica and 740–1280 ng/kg in Robusta green coffee) is most likely of microbial origin.

Sesquiterpenic alcohols Examples of sesquiterpenic alicyclic alcohols (**8-24**) are farnesol and nerolidol (also known as peruviol). Both alcohols, smelling of flowers, are components of many essential oils used in perfumery. Of the four possible geometric isomers, the (2*E*,6*E*)-isomer of farnesol, is the most common in nature and occurs, for example, in basil oil and ambrette (*Abelmoschus moschatus*; Malvaceae) seed oil. The (2*Z*,6*E*)-isomer occurs in the petit grain oil bigarade, which is derived from the bitter orange tree leaves (*Citrus aurantium* var. *amara*, Rutaceae). Farnesol is a natural pesticide for mites and a pheromone for several species of insects. Nerolidol with a double bond at C-6 occurs in the form of (*Z*)- and (*E*)-isomers, each of which can exist as an enantiomeric pair (chiral carbon C-3). The individual enantiomers and their mixtures are found in many essential oils. For example, the essential oil known as cabreuva oil that is used in perfumery is obtained from the bark of the South American tree *Myrocarpus frondosus* (Fabaceae) and contains the (+)-(*E*)-isomer of nerolidol (3*S*,6*E*)-nerolidol. (*S*)-Nerolidol (in some plants) and in part (*R*)-nerolidol are precursors of acyclic hydrocarbons (*E*)-4,8-dimethylnona-1,3,7-triene and (3*E*,7*E*)-4,8,12-trimethyltrideca-1,3,7,12-tetraene, which are formed by oxidative degradation. These hydrocarbons are released from the damaged leaves of many plants, but they are also typical components of the scent emitted by some plants (belonging to the Orchidaceae, Cactaceae, Magnoliaceae and Liliaceae families) that bloom at night.

An example of monocyclic sesquiterpenic alcohols is (+)-α-bisabolol (**8-24**). It occurs in citrus essential oils along with other sesquiterpenic alcohols, and represents the major component of the essential oil of chamomile flowers (*Matricaria chamomilla*, Asteraceae). α-Bisabolol (0.26%) and other sesquiterpenoids, such as monocyclic sesquiterpenic alcohol (*Z*)-lanceol (1.7%), bicyclic sesquiterpenic alcohol (*Z*)-α-*trans*-bergamotol (3.7%), (−)-(*Z*)-β-santalol (about 21%) and its isomers are the major and essential components of white sandalwood oil (*Santalum album*, Santalaceae) used in perfumery, cosmetics and aromatherapy. (*Z*)-Lanceol is also a component of sage species (*Salvia* spp., Lamiaceae) essential oil. Sesquiterpenic bicyclic alcohol (+)-β-nootkatol is the precursor of ketone (+)-nootkatone, an important component of grapefruit essential oil. Trivial and systematic names of the main sesquiterpenic alcohols are listed in Table 8.7.

Many sesquiterpenoids have antimicrobial and insecticidal properties. For example, germacrene A is the precursor of capsidiol (see



8-24, sesquiterpenic alcohols

Table 8.7 Trivial and systematic names of selected sesquiterpenic alcohols.

Trivial name	Systematic name (IUPAC)
(*Z*)-α-*trans*-Bergamotol	(1*S*,3*E*,5*S*)-4-Hydroxymethyl-7-methyl-7-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-3-ene
(−)-α-Bisabolol	(*S*)-6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hept-5-en-2-ol
(*E*,*E*)-Farnesol	(2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-ol
(−)-Geosmin	(4*S*,4a*S*,8a*R*)-4,8a-Dimethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-4a-ol
(+)-Hernandulcin	(2*S*,6*S*)-6-(2-Hydroxy-6-methylhept-5-en-2-yl)-3-methylcyclohex-2-en-1-one
Capsidiol	(1*R*,3*R*,4*S*,4a*R*,6*R*)-4,4a-Dimethyl-6-prop-1-en-2-yl-1,2,3,4,5,6,7-heptahydronaphthalen-1,3-diol
(*Z*)-Lanceol	(*Z*)-6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-7-ol
(+)-(*Z*)-Nerolidol	(3*S*,6*Z*)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol
(+)-(*E*)-Nerolidol	(3*R*,6*E*)-3,7,11-Trimethyldodeca-1,6,10-trien-3-ol
(+)-Nootkatol	(2*R*,4*S*,4a*S*,6*R*)-4,4a-Dimethyl-6-prop-1-en-2-yl-2,3,4,5,6,7,8-heptahydronaphthalen-2-ol
(−)-(*Z*)-β-Santalol	(1*S*,2*Z*,4*R*,6*R*)-2-Methyl-5-[6-methyl-5-methylidene-6-bicyclo[2.2.1]heptanyl]pent-2-en-1-ol

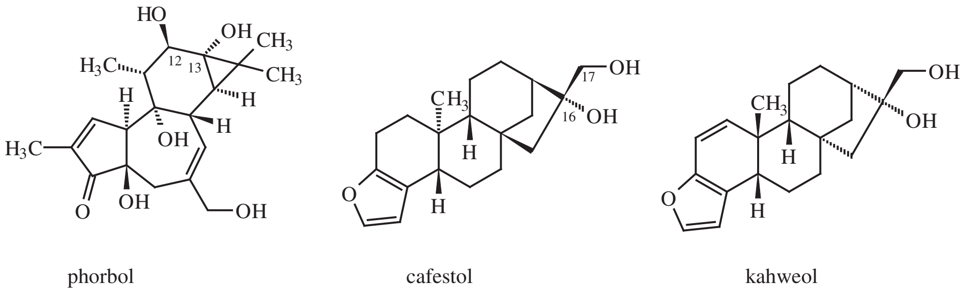
10-155), which is the main phytoalexin that forms in bell peppers and tobacco plants, when attacked by pathogenic fungi. Unusual sesquiterpenoids are sweet hydroxy ketones (+)-hernandulcin and (+)-4β-hydroxyhernandulcin (see **11-35**). Many other sesquiterpenoids have a bitter taste (see Section 8.3.4).

An unusual sesquiterpenoid (though it has only 12 carbon atoms) is (−)-geosmin (**8-23**), produced by some microorganisms, including cyanobacteria and actinobacteria, especially species of the genus *Streptomyces*. Geosmin causes off-flavour in drinking water and some foods. For example, it is responsible for the characteristic earthy flavour of beetroot (cultivars of *Beta vulgaris*, Amaranthaceae, formerly Chenopodiaceae) during cooking. Biosynthesis of alcohol geosmin starts from the sesquiterpenic hydrocarbon germacrene D, but during the biosynthesis three carbon atoms are eliminated as acetone. A similar off-flavour may be caused by monoterpenic alcohol (−)-2-methylisoborneol (**8-22**).

Diterpenic alcohols Diterpenic alcohols are present in nature as free compounds or bound in fatty acid esters and glycosides. They have no significance as flavourings, but may be precursors of flavour-active products. A number of diterpenoids are biologically active substances. Diterpenic alcohols known as gibberellins are found universally in plant tissues, and act as plant hormones.

An important representative of diterpenoid alcohols is phytol (2*E*,3,7*R*,11*R*,15)-3,7,11,15-tetramethylhexadec-2-en-1-ol, a constituent of chlorophylls, tocopherols and K group vitamins. Free phytol arises during chlorophyll hydrolysis by chlorophyllase as an integral part of plant catabolism in fruit ripening and yellowing of leaves. Microbial decomposition of phytol creates some unusual branched fatty acids, such as pristanic and phytanic acids (see Section 3.2.3.3.2). Acyclic diterpenic alcohol is also retinol (vitamin A_1) (see Section 5.2.1).

Many diterpenoid alcohols are cyclic compounds occurring as free substances, but also as fatty acid esters or glycosides. The toxicity of latex of many plant species from the spurge family (Euphorbiaceae) is caused by the presence of phorbol esters such as 13-acetyl-12-myristoyl-phorbol. Cafestol, kahweol (**8-25**) and related diterpenoids (16-*O*-methylcafestol, 16-*O*-methylkahweol and other compounds) are found in green coffee (*Coffea* spp., Rubiaceae) beans (mainly esterified to fatty acids at the C-16 or C-17 position) and unfiltered coffee prepared from roasted coffee seeds, such as Turkish coffee. Kahweol is specific to *C. arabica* coffee, where it occurs in concentrations of about 5890 mg/kg of fresh weight and 5200 mg/kg in the endosperm and perisperm, respectively, while 16-*O*-methylcafestol occurs only in robusta coffee (*C. canephora*). The amount of cafestol in *C. arabica* is about



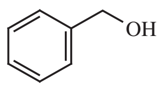
8-25, diterpenic alcohols

3000 mg/kg in the endosperm and 1300 mg/kg in the perisperm and in *C. canephora* the amounts are about 940 mg/kg and 1100 mg/kg, respectively. Cafestol (and also to some extent kahweol) increases the cholesterol level in the blood serum as a result of interaction of palmitate with biological membranes. Furthermore, anticarcinogenic, antioxidant and anti-inflammatory properties as well as hepatoprotective effects have been reported. During roasting of green coffee seeds, these alcohols partially dehydrate to dehydroalcohols (dehydrocafestol and dehydrokahweol) and are oxidised to the corresponding aldehydes (cafestal and kahweal).

Teucrin A is the major component of the so-called *neo*-clerodane diterpenoids (**8-26**) from the plant known as wall germander (*Teucrium chamaedrys*, Lamiaceae), native to Europe and the Near East and used in the form of alcoholic extracts for the aromatisation (bittering) of wines and aperitifs (as it contains a number of odour-active mono- and sesquiterpenoids), and in folk medicine for its antiseptic (anti-inflammatory) and choleretic (increased bile excretion) properties. Teucrin A also occurs in some other species of the genus *Teucrium* and is accompanied by further diterpenoids, such as teuscorolide, teuquin, teuflin (**8-26**) and their glycosides. Because of its hepatotoxicity, teukrin A is in the list of substances of Regulation (EC) No. 1334/2008, which must not be added to foods as flavourings. Its natural content is restricted to 5 mg/kg in bitter-tasting spirit drinks or bitters and to 2 mg/kg in other alcoholic beverages.

8.2.2.1.2 Aromatic and heterocyclic alcohols

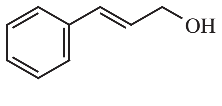
Aromatic alcohols are natural constituents of many essential oils. They arise as secondary compounds in fermentation and thermal processes. The simplest alcohol in this group is benzyl alcohol (**8-27**). It is assumed that benzyl alcohol results from the gradual reduction of benzoic acid by dehydrogenase enzymes via benzaldehyde. In alcoholic beverages it is produced by reduction of benzaldehyde, which is a product of cyanogenic glycosides decomposition and a byproduct of Strecker degradation of phenylalanine. In fruit brandies produced from fermenting fruits with stones (such as cherry, apricot and plum brandies), larger quantities amounting to 20–70 mg/l of benzyl alcohol are found. Benzyl alcohol also occurs in the form of esters in some essential oils. The most common compounds are benzyl acetate and benzyl benzoate.



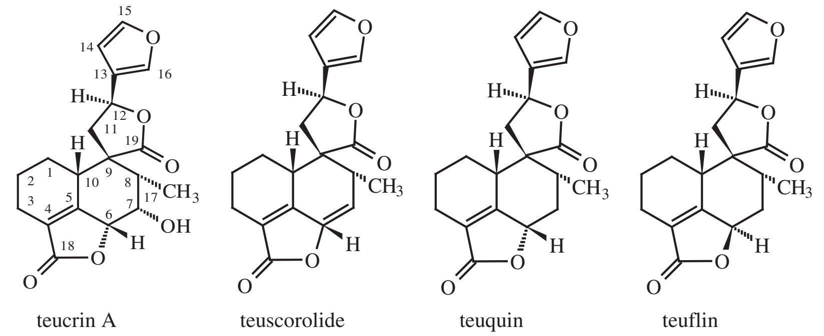
8-27, benzyl alcohol

Common non-volatile components of plants are hydroxy derivatives of benzyl alcohol that mainly occur in nature in the bound form as glycosides, esters of aromatic carboxylic acids (caffeic, protocatechuic, 4-hydroxybenzoic and vanillic acids) or glycosides of these esters. For example, 4-hydroxybenzyl alcohol was first isolated from muskmelon seedlings (*Cucurbita moschata*). The compound acts as a cofactor for indoleacetic acid oxidase. The glycoside of its ester with protocatechuic acid, 4-(3,4-dihydroxybenzoyloxymethyl)phenyl-*O*-β-D-glucopyranoside occurs in oregano (*Origanum vulgare*), which has been reported to possess antithrombin, anti-*Helicobacter pylori*, antibiotic, antihyperglycaemic and antioxidation effects.

During fermentation processes, phenylalanine produces the higher homologue of benzyl alcohol 2-phenylethanol, also known as phenylethyl alcohol (**8-12**). Analogously, tyrosol results from tyrosine (**8-12**), hydroxytyrosol from 3,4-dihydroxyphenylalanine (DOPA; **8-12**) and tryptophol from tryptophan (**8-12**), but these aldehydes are non-volatile compounds. 2-Phenylethanol formed by reduction of phenylacetaldehyde is one of the main components of rose oil and occurs in small quantities in many other essential oils. One component of cinnamon oil (see Table 8.32, later), fruit brandies and other materials is the *trans*-isomer of cinnamyl alcohol, (*E*)-cinnamyl alcohol (**8-28**), which is produced by the reduction of cinnamic acid via cinnamic acid aldehyde (cinnamaldehyde). In the form of various esters, cinnamyl alcohol is found in the leaves and bark of evergreen aromatic trees and shrubs of the genus *Cinnamomum* (Lauraceae), growing in tropical and subtropical regions of America, Asia and Australia, and in Peru balsam, secretion of trees of the *Myroxylon balsamum* (Fabaceae)



8-28, (*E*)-cinnamyl alcohol



teucrin A teuscorolide teuquin teuflin

8-26, *neo*-clerodane diterpenoids

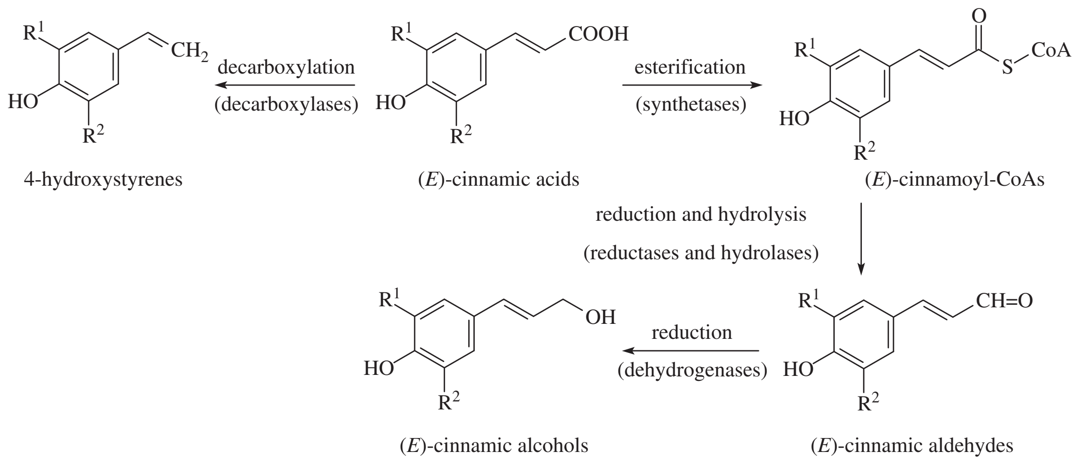


Figure 8.10 Biosynthesis of cinnamyl alcohol from cinnamic acid.

native to Central and South America, which is used in perfumery. A number of related substituted alcohols derived from substituted cinnamic acids, such as 4-coumaryl, caffeoyl, coniferyl (feruloyl) and 5-hydroxyconiferyl (sinapoyl or sinapyl) alcohol, and the corresponding aldehydes, are building units of lignin (Figure 8.10).

The most common oxygen-containing heterocyclic alcohol is furfuryl alcohol, which is a degradation product of sugars. Furfuryl alcohol results primarily from furan-2-carbaldehyde by reduction or a Cannizzaro reaction. A large number of other alcohols derived from furan, pyran and other heterocyclic compounds are products of the Maillard reaction.

8.2.2.1.3 Glycols and polyols

Glycols and polyols are non-volatile compounds that are formed in foods as secondary products of fermentation processes and of the Maillard reaction, or may be used as food additives. Frequently occurring polyols present in foods are sugar alcohols, which act as taste-active substances.

The lowest member of the homologous series of glycols is ethylene glycol. It may be released to the environment as a contaminant, with the major source being the disposal of used antifreeze and deicing solutions. It is also used in hydraulic brake fluids, inks in stamp pads and ball point pens. Another two-carbon compound is the oxidation product of ethylene glycol glycolaldehyde that is produced as a degradation product of sugars in the Maillard reaction.

The three-carbon compound methylglyoxal is the byproduct of fermentation processes and of the Maillard reaction. Products of its reduction, namely acetol (hydroxyacetone), (*R*)-propane-1,2-diol (D-propane-1,2-diol) and (*R*)-lactaldehyde (D-lactic acid aldehyde or D-lactaldehyde) are relatively common compounds present in small amounts in foods (Figure 8.11). Under anaerobic conditions, some microorganisms (such as *Bacillus amaracrylus*, *Citrobacter freundii* or *Klebsiella pneumoniae*) can ferment glycerol as the sole substrate and transform it into 1,3-dihydroxyacetone (which is then involved in metabolic pathways) or 3-hydroxypropionaldehyde (by a coenzyme B_{12}-dependent glycerol dehydratase), which was first discovered in wine spoiled by *B. amaracrylus*. It is further reduced to propane-1,3-diol. 3-Hydroxypropionaldehyde dimer (see Section 4.7.1.2.3) was patented under the name reuterin and is thought to be responsible for the probiotic effects of lactic acid bacteria *Lactobacillus reuteri*. Accordingly, the food industry has begun industrial applications with *L. reuteri* to enhance the quality and value of milk products. Bacteria *L. brevis* can produce propane-1,3-diol from glycerol as a product of sugar and lactic acid co-fermentation.

Commercial propane-1,2-diol (propylene glycol) is a racemic mixture of both stereoisomers. It is used to absorb excess water, if used as a humectant, and to maintain moisture in some medicines, cosmetics and foods, and as a solvent for food colourings and stabilisers of carbonyl compounds in food flavours. It is also used in antifreeze and de-icing solutions and as a solvent in the paint and plastic industries.

The most important diols occurring in alcoholic beverages and fermented dairy products are butane-2,3-diol and pentane-2,3-diol that are not, however, odour-active substances. These diols are produced as byproducts due to the activity of certain microorganisms, along with the corresponding acyloins (acetoin and 3-hydroxypentane-2-one) and sensory active α-diketones (biacetyl and pentane-2,3-dione).

In fermented dairy products, such as yoghurt and butter, all these four-carbon compounds are produced from pyruvic acid, which is

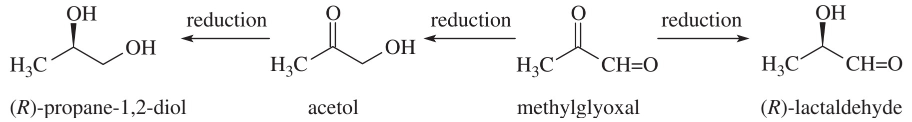
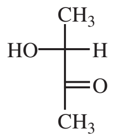
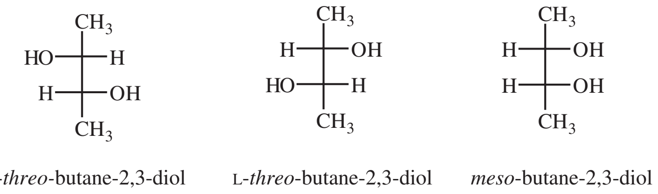


Figure 8.11 Formation of acetol, lactaldehyde and propane-1,2-diol.

formed from citric acid. In the so-called malolactic fermentation of wine, pyruvic acid arises mainly by decarboxylation of (*S*)-malic (L-malic) acid. The conversion of pyruvic acid into lactic acid (catalysed by L-lactate dehydrogenase and D-lactate dehydrogenase) requires NADH (nicotinamide adenine dinucleotide, see Section 5.8.2). If NADH is not available, some lactic acid bacteria (such as *Streptococcus*, *Leuconostoc*, *Lactobacillus*, *Pediococcus* and *Oenococcus*; in fermented dairy products lactic acid bacteria *Streptococcus diacetylactis* are important and in wines, bacteria *Oenococcus oeni*) transform most of the pyruvic acid and acetaldehyde, which is generated by its decarboxylation, to an unstable intermediate (*S*)-2-hydroxy-2-methyl-3-oxobutanoic acid, also known as (*S*)-2-acetolactic acid (Figure 8.12). Decarboxylation and oxidation of 2-acetolactic yields provides butane-2,3-dione (biacetyl), while decarboxylation yields (*R*)-3-hydroxybutan-2-one (acetoin, **8-29**), and reduction of acetoin gives (2*R*,3*R*)-butane-2,3-diol, also known as (−)-D-*threo*-butane-2,3-diol (**8-30**). In small quantities, it is accompanied by (2*S*,3*S*)-butane-2,3-diol, also known as (+)-L-*threo*-butane-2,3-diol, and *meso*-butane-2,3-diol (**8-30**). The amount of the *meso* isomer is 20–38% of the total amount of butane-2,3-diol. Not all lactic acid bacteria produce all of these compounds, and in some cases the reaction stops earlier and some intermediates accumulate. Acetic acid is also a fermentation product.



8-29, acetoin



8-30, butane-2,3-diol isomers

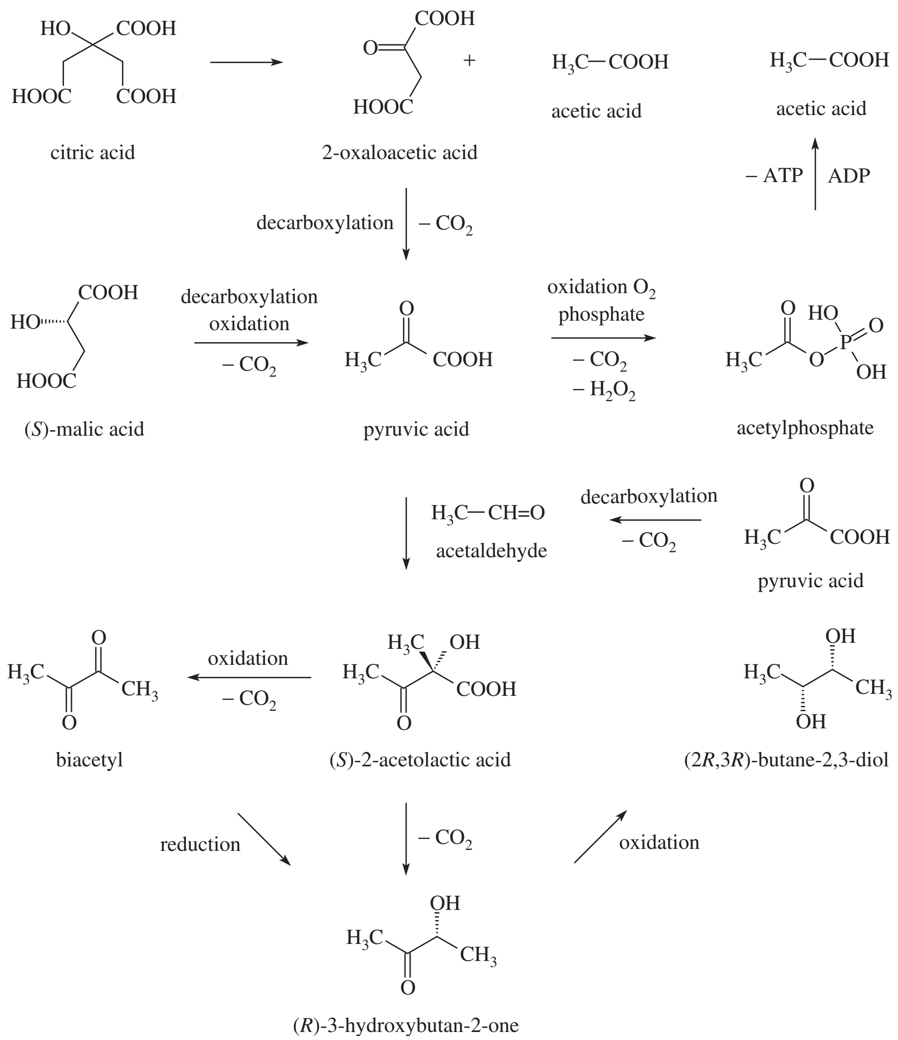


Figure 8.12 Formation of butane-2,3-diol, acetoin and biacetyl.

Five-carbon compounds are formed from (*S*)-aceto-2-hydroxybutyric acid, also known as (*S*)-2-ethyl-2-hydroxy-3-oxobutanoic acid, which is the intermediate of isoleucine biosynthesis from threonine. This acid is formed in the reaction of 2-oxobutanoic acid with acetaldehyde and its decarboxylation provides (*R*)-3-hydroxypentane-2-one, which is oxidised to pentane-2,3-dione and reduced to (2*R*,3*R*)-pentane-2,3-diol (Figure 8.13).

Glycerol (propane-1,2,3-triol) mainly occurs in foods in the form of triacylglycerols, and many other lipids (glycerolipids). Glycerol is also produced as a byproduct of alcoholic fermentation (also in alkaline media together with acetic acid salts) by reduction of the intermediate 1,3-dihydroxyacetone phosphate via glycerol 3-phosphate.

Glycerol is often found in beer, wine and other alcoholic beverages. The amount of glycerol depends mainly on the temperature during fermentation, yeast strain and, in wine, on the presence of sulfites. Glycerol concentrations in Pilsner-type beers are 1.5–2.9 g/l (in top-fermented beers). In wine, levels between 1 and 15 g/l are frequently encountered, with average values of approximately 7 g/l. Higher amounts of glycerol result from higher temperatures, so its content in red wines is usually higher (by about 20–30%) than the content in white wines. In the presence of higher amounts of sulfites, yeast *Saccharomyces cerevisiae* produces virtually no alcohol, because acetaldehyde is blocked in the form of the α-hydroxysulfonic acid, and cannot be reduced to ethanol. However, 1,3-dihydroxyacetone phosphate is still reduced to glycerol 3-phosphate, which is hydrolysed to glycerol. A higher glycerol content is also found in wines produced from grapes infected with the *Botrytis cinerea* fungus. Glycerol affects the organoleptic properties of beer and wine. Higher levels are thought to contribute to the viscosity, sweetness and smoothness of beverages.

8.2.2.2 Properties and reactions

Low molecular weight alcohols and glycols are toxic. Methanol and ethanol are both biotransformed by alcohol dehydrogenase; however, ethanol has the greater affinity for the enzyme. The toxicity resulting from higher doses of methanol is very well documented in both humans and animals and is attributed to its toxic metabolite formic acid, which is known to be toxic to the optic nerve. Formic acid requires folic acid as a cofactor for its elimination. Animal studies have shown that when folate levels are low, the elimination of formic acid is slower. Therefore, folate deficient chronic drinkers may be at higher risk of organ damage, blindness and even death. The lethal oral dose of methanol is 340 mg/kg body weight. Levels of methanol in fruit brandies are usually around 4 g/l, but can be much higher (e.g. 12 g/l in plum, apple, pear, raspberry, blackberry brandies and 2 g/l in wine brandies). The current EU general limit for naturally occurring methanol is 10 g of methanol per litre of ethanol (which equates to 0.4% v/v of methanol in a 40% alcohol beverage) – this provides a safety margin.

Ethanol acts on the central nervous system, causing euphoria at the beginning, but severe poisoning can lead to death. Ethanol is an addictive poison that causes alcoholism (ethylism). Ethylene glycol is a sweet toxic compound that can damage the kidneys, nervous system, heart and lungs.

As in water, hydrogen bonds also play the major role in the molecular attractive forces in alcohols (especially in lower alcohols

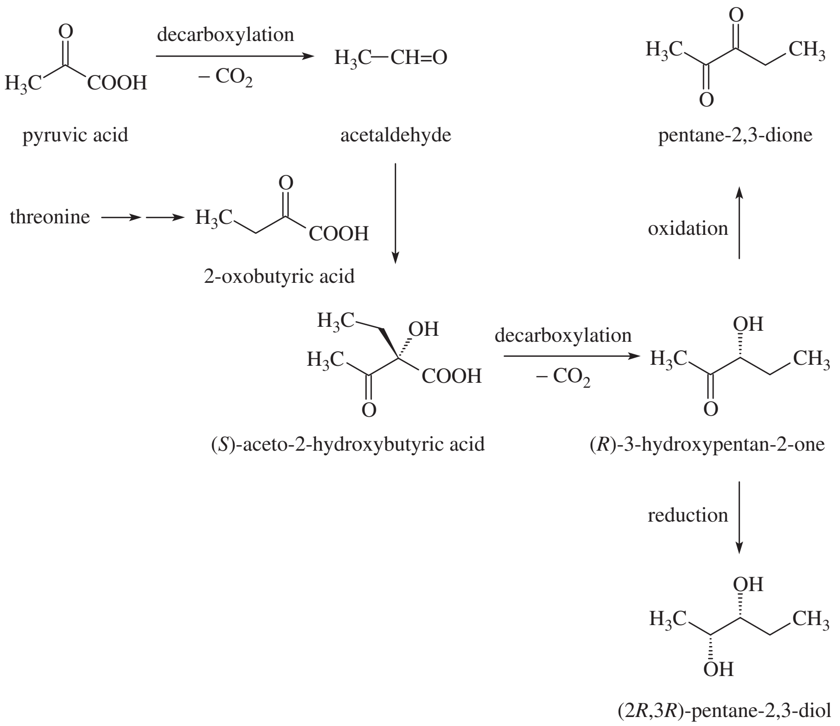


Figure 8.13 Formation of pentane-2,3-diol, 3-hydroxypentan-2-one and pentan-2,3-dione.

and polyols). This is related to the relatively good solubility of alcohols and glycols in water, and to their relatively high boiling points. The large number of hydroxyl groups allows polyols to create complex molecular associates through hydrogen bonding in solutions.

The most important reactions taking place on the hydroxy groups of alcohols are O–H bond cleavage and C–O bond cleavage. With the O–H bond cleavage, reactions with strong acids proceed, as do oxidations of primary alcohols to aldehydes, secondary alcohols to ketones and reactions with organic acids (formation of esters). In foods the last three reactions are particularly important, and are usually enzymatically catalysed. Other important reactions are dehydration and the opposite reaction, hydration, which yield unsaturated hydrocarbons from alcohols and isomeric alcohols from unsaturated hydrocarbons, respectively. These reactions are particularly important in terpenic alcohols. In oleochemistry, oxidation and esterification reactions are used for the production of various lipid derivatives.

8.2.3 Ethers

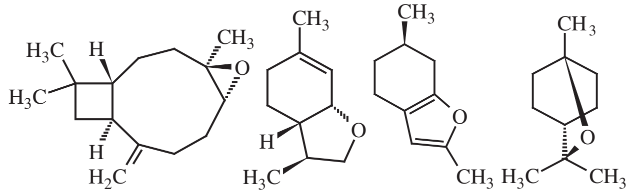
Symmetric and asymmetric aliphatic, alicyclic and aromatic ethers and ethers with the oxygen atom bound in the ring can be found in foods. Volatile dialkyl ethers are virtually absent from foods, but some are synthesised and used as flavourings, especially for cosmetic purposes. Odour- and taste-active ethers are mainly terpenoid ethers, derived from monoterpenes and sesquiterpenes, and aromatic ethers.

8.2.3.1 Classification structure, terminology and occurrence

8.2.3.1.1 Terpenoid ethers

Ethers, whose oxygen atom is bound to vicinal carbons or is part of a larger alicyclic ring, include 1,2-epoxides (oxiranes), 1,4-epoxides (furans) and 1,5-epoxides (pyrans). These and other terpenic epoxides are the primary components responsible for the odour of many foods. Terpenoid ethers also form as secondary products of the oxidation and dehydration of carotenoid pigments, steroids, fatty acids, polycyclic aromatic hydrocarbons and many other compounds.

An example of terpenic 1,2-epoxides is β-caryophyllene oxide, also known as (−)-epoxycaryophyllene (**8-31**), which occurs in many essential oils. An example of terpenic 1,4-epoxides is the so-called (+)-dill ether, (*3R*,4*S*,8*S*)-3,9-epoxy-*p*-menth-l-ene (**8-31**), which is a typical component of the essential oil of caraway (30%) and dill. An example of unsaturated 1,4-epoxides is (+)-menthofuran (**8-31**), the metabolite of ketone (+)-pulegone. Both compounds are components of peppermint oil (see Table 8.32, later) and are hepatotoxic. Monoterpenoid compound (+)-1,8-cineole (also known as limonene oxide, eucalyptol or 1,8-epoxy-*p*-menthane; **8-31**) is an example of more complex structures. It is present in essential oils of many types of spices, and higher quantities are found in the essential oil of trees of the genus *Eucalyptus* (Myrtaceae). Trivial and systematic names of selected ethers are given in Table 8.8.



epoxycaryophyllene dill ether menthofuran 1,8-cineole

8-31, terpenoid epoxides

Numerous other ethers containing pyran or furan rings are formed by the dehydration of aliphatic diols (e.g. linalool oxides, rose oxide or nerol oxide) and are components of many essential oils. For example, the furanoid (2*R*,5*R*)-(*E*)- and (2*R*,5*S*)-

Table 8.8 Trivial and systematic names of some terpenoid ethers.

Trivial name	Systematic name (IUPAC)
(−)-Epoxycaryophyllene	(1*R*,4*R*,6*R*,10*S*)-9-Methylene-4,12,12-trimethyl-5-oxatricyclo[8.2.0.0^{4,6}]dodecane
(+)-Dill ether	(3*S*,3a*S*,7a*R*)-3,6-Dimethyl-2,3,3a,4,5,7,7a-hexahydro-1-benzofuran
(+)-Menthofuran	(*R*)-3,6-Dimethyl-4,5,6,7-tetrahydro-1-benzofuran
(+)-1,8-Cineol	(1*S*,4*S*)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane
(−)-Nerol oxide	(*S*)-3,6-Dihydro-4-methyl-2-(2-methylprop-1-enyl)-2*H*-pyrane
(−)-(*Z*)-Rose oxide	(2*S*,4*R*)-4-Methyl-2-(2-methylprop-1-enyl)oxane
(2*R*,5*S*)-(*Z*)-Linalool oxide (furanoid)	(2*R*,5*S*)-2-methyl-5-prop-1-en-2-yl-2-vinyltetrahydrofuran
(2*R*,5*R*)-(*E*)-Linalool oxide (furanoid)	(2*R*,5*R*)-2-methyl-5-prop-1-en-2-yl-2-vinyltetrahydrofuran
(3*R*,6*R*)-(*Z*)-Linalool oxide (pyranoid)	(3*R*,6*R*)-2,2,6-Trimethyl-6-vinyltetrahydro-2*H*-pyran-3-ol
(3*S*,6*S*)-(*Z*)-Linalool oxide (pyranoid)	(3*S*,6*S*)-2,2,6-Trimethyl-6-vinyltetrahydro-2*H*-pyran-3-ol

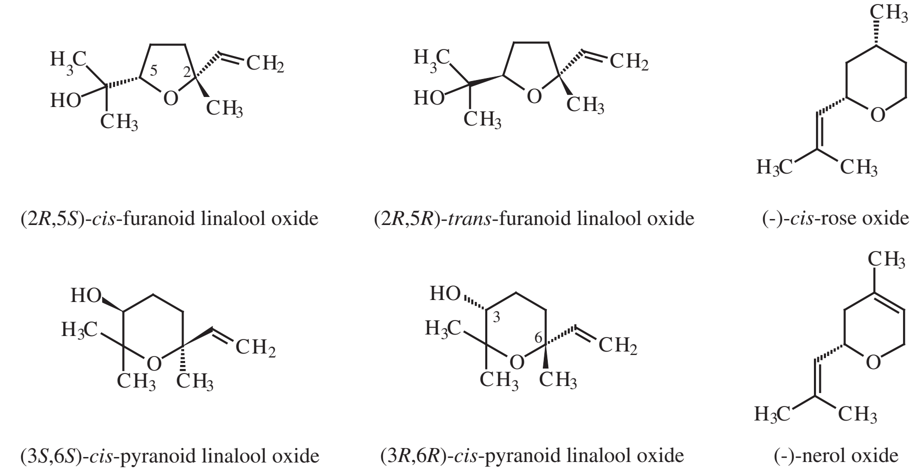
(*Z*)- and pyranoid (3*R*,6*R*)-(*Z*)- and (3*S*,6*S*)-(*Z*)-linalool oxides (**8-32**) are odorants of jasmine tea, aromatic wines, elderberry bush flowers and linden honey. In grapes, (−)-(*Z*)-rose oxide from (−)-citronellol is analogously produced. Rose oxide is also a component of rose and geranium essential oils (**8-32**). Nerol oxide in rose oil is a racemate.

8.2.3.1.2 Aromatic ethers

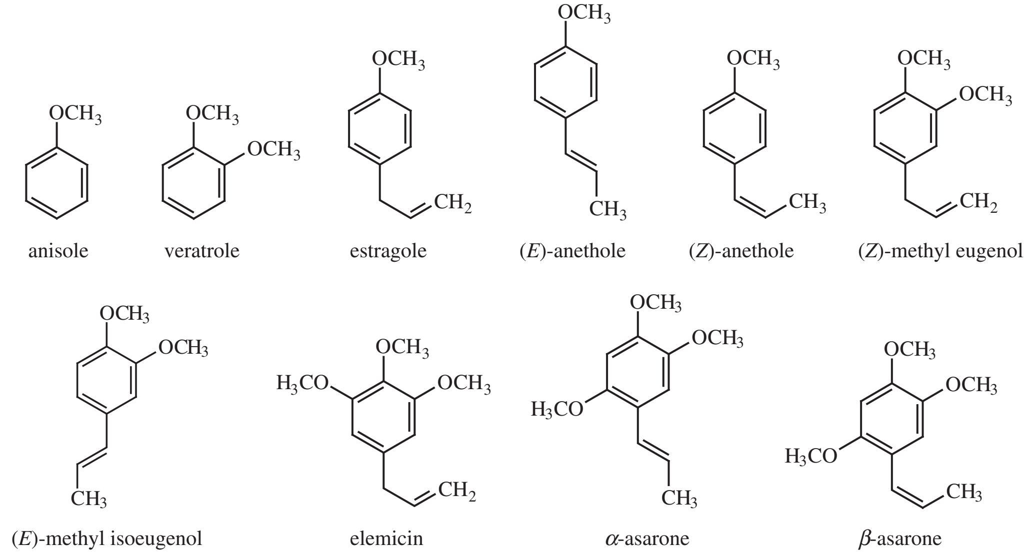
Foods may contain a number of alkyl aryl ethers that are components of essential oils of different spices as well as secondary substances. These ethers are most often derived from anisole (methoxybenzene, **8-33**) or veratrole (1,2-dimethoxybenzene, **8-33**), which are substituted by a prop-1-en-yl or 1-prop-2-en-1-yl (allyl) group at the C-4 position of the benzene ring. An important ether is estragole (also known as 4-allylanisole or methyl chavicol, **8-33**), which is the main component (over 80%) of basil essential oil (see Table 8.32, later) and tarragon (dragon's wort) essential oil (more than 60%). Estragole is a genotoxic carcinogen in experimental animals after chronic exposure, and therefore appears in the list of substances of Regulation (EC) No. 1334/2008, which shoud not be added to foods as flavourings. Its natural content in foods is restricted to 50 mg/kg in dairy products, processed fruits, vegetables including mushrooms (roots, tubers and legumes), nuts and seeds and fish products and to 10 mg/kg in non-alcoholic beverages.

Isomers of anethole (isoestragole, **8-33**) with a typical anise aroma are the main components of anise (more than 95%), fennel (more than 80%) and star anise (more than 95%) essential oils. In natural essential oils the (*E*)-isomer of anethole dominates, which is used as a flavour and fragrance agent as it has a liquorice-type odour and an anise-type taste. The (*Z*)-isomer of anethole has an anise-type odour. It was associated with liver cancer in rats, but is now regarded as safe.

Components of clove oil (see Table 8.32, later) and many other oils are β-caryophyllene (4–21%), eugenol (49–87%), eugenyl acetate (0.5–21%), methyleugenol and elemicin (**8-33**). Another



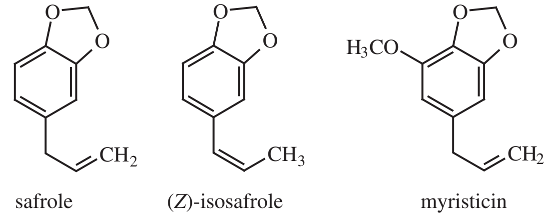
8-32, monoterpenoid oxides



8-33, aromatic ethers

compound derived from veratrole is methylisoeugenol, which can occur as both (*E*)- and (*Z*)-isomers (**8-33**). (*E*)-Methylisoeugenol occurs in high concentrations in the seed oil and oil obtained from aerial parts of carrot (*Daucus carota*), which is reported to be an antimicrobial against the human enteropathogen *Campylobacter jejuni*. Methylisoeugenol is found in lower amounts in many other essential oils (such as citronella, calamus, nutmeg and laurel leaf oils). Elemicin is also a component of carrot essential oil and banana aroma. A component of the essential oil obtained from the root of calamus, also known as sweet flag (*Acorus calamus*, Acoraceae), and occurring in some other plants (such as European wild ginger, *Asarum europaeum*, Aristolochiaceae), is asarone. It is present as a mixture of two isomers, of which α-asarone (**8-33**) is the (*E*)-isomer and β-asarone (**8-33**), also known as *cis*-isoasarone, is the (*Z*)-isomer. Calamus root is used as a medicinal plant for wound healing, as an antipyretic drug, against dyspepsia (indigestion, upset stomach), as well as a bittering agent for alcoholic beverages (including vermouth and beer) and in cosmetics (such as flavourings for toothpastes). β-Asarone has antifungal activity and completely inhibits mycelial growth of some plant pathogenic fungi, *Cladosporium cucumerinum*, *Colletotrichum orbiculare*, *Magnaporthe grisea* and *Pythium ultimum*, but also has toxic effects in mammals (it acts as a chemosterilant) and therefore appears in the list of substances of Regulation (EC) No. 1334/2008, which should not be added to foods as flavourings. Its natural content in alcoholic beverages is limited at a maximum level of 1 mg/kg and tetraploid forms of the plant should not be used for the production of flavourings and food ingredients with flavouring properties. A related North American species *A. americanus*, today described as a variety of calamus (*A. c.* var. *americanus*), does not contain β-asarone at all. β-Asarone and related 2,4,5-trimethoxybenzaldehyde have also been found in carrot seeds.

Methyleugenol (**8-33**), a component of several essential oils of, for example, fennel, citronella, basil, bay and tea tree (*Melaleuca* spp.), as well as eugenol and asarons are related to compounds with an attached 1,3-dioxol ring (methylenedioxy group). Most substances are simple allyl or prop-1-en-1-yl benzenes, substituted by methoxyl groups. Important compounds are safrole (1-allyl-3,4-methylenedioxybenzene, **8-34**), isosafrole, 1-(prop-1-en-1-yl)-methylene-3,4-dioxybenzene (**8-34**) and myristicin, also known as 5-methoxysafrole (1-allyl-5-methoxy-3,4-methylenedioxybenzene, **8-34**). Myristicin is a characteristic component of essential oils from the seeds of some root vegetables (carrots, parsley and celery) and herbs, in particular, of essential oils derived from nutmeg and mace (see Table 8.32, later). Safrole is the main component



8-34, aromatic ethers with a methylenedioxyphenyl moiety

(representing more than 80%) of sassafras oil (shikimol) derived from the root bark or the fruit of the sassafras tree (*Sassafras albidum*, Lauraceae) growing in North America, which was used in the United States to flavour beer and soft drinks until 1978. Locally, sassafras herbal tea is still in use in the treatment of rheumatism and skin diseases. Safrole is also a component of essential oils of nutmeg and mace (the content is about 0.1%) and of anise, cinnamon and some other essential oils. Isosafrole is also a common component of many essential oils (such as sassafras, laurel and clove essential oils).

Methyleugenol and safrole exhibit toxic effects of weak carcinogens in experimental animals. Myristicin is a psychomimetic compound tending to induce narcotic effects, hallucinations and other symptoms of a psychosis. Methyleugenol and safrole are in the list of substances of Regulation (EC) No. 1334/2008, which should not be added to foods as flavourings. The natural content of methyleugenol in foods (the maximum level of safrole is in parenthesis) is restricted to 60 (25) mg/kg in soups and sauces, to 20 mg/kg in dairy products and ready-to-eat savouries, to 15 (15) mg/kg in meat preparations and meat products, including poultry and game, to 10 (15) mg/kg in fish preparations and fish products and to 1 (1) mg/kg in non-alcoholic beverages. Sassafras leaves must be safrole-free to be used as food additives, and sassafras oil is not used at all.

8.2.3.1.3 Other ethers

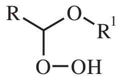
Simple oxiranes (e.g. oxirane, known as ethylene oxide, and methyloxirane, known as propylene-1,2-oxide) are used as food additives (such as preservatives). Some ethers can also be regarded as phenols, aromatic aldehydes (such as vanillin) or acetals. Certain acetals derived from aliphatic aldehydes are used as fragrances.

Particular non-volatile ethers are classified as lipids (e.g. plasmalogens, esters of 1-alkoxypropan-2,3-diols, such as chimyl alcohol). Some ethers also occur in small amounts as substances accompanying lipids. Examples of these compounds are dialicyclic ethers derived from sterols that occur in refined vegetable oils. Of great importance in oleochemistry are non-volatile ethers of glycerol and other polyols (dimers and higher oligomers) that are used, for example, as emulsifiers. The so-called vinyl ethers are formed as secondary oxidation products of essential fatty acids, representatives of which are pentylfuran and two isomeric 2-(pent-2-en-1-yl)furans that cause an off-flavour of some refined vegetable oils, known as reversion.

Dehydration of carbohydrates in acidic solution yields derivatives of furan-2-carbaldehyde. An example of a heterocyclic ether is the ether derived from 5-hydroxymethylfuran-2-carbaldehyde that accompanies the parent aldehyde in sugar hydrolysates. Furfuryl ethyl ether is an important flavour compound indicative of beer storage and aging conditions. It is most likely formed by protonation of furfuryl alcohol or furfuryl acetate followed by substitution of the leaving group by the nucleophilic ethanol. Another example of a heterocyclic ether is the ether formed by dehydration of 3-hydroxymethylindole in cruciferous vegetables, where 3-hydroxymethylindole arises by degradation of glucosinolate glucobrassicin.

8.2.3.2 Properties and reactions

Ethers are relatively stable in acidic and alkaline media, but they are easily oxidised to the corresponding hydroperoxides (**8-35**) that are often transformed into thermolabile polymeric peroxides.



8-35, ether hydroperoxide (α-hydroperoxyoxaalkane)

8.2.4 Carbonyl compounds

8.2.4.1 Classification, structure, terminology and occurrence

The carbonyl compounds molecules contain either an aldehyde group –CH=O or a keto group (oxo group) –C(=O)–, therefore carbonyl compounds can be divided into:

- aldehydes
- ketones.

Volatile aldehydes and ketones are the most important odour- and taste-active substances. They occur in foods as primary substances, as components of various essential oils and also result from enzymatic and chemical reactions from various precursors as secondary substances. They are often desirable flavour-active components of foods, but in some cases may also carry undesirable odour and taste. Then they serve as indicators of unwanted changes in sensory or nutritional value of foods (such as autoxidation of lipids).

Carbonyl compounds also include a range of non-volatile polar compounds, such as reducing sugars or some products of their transformation (degradation), which are often taste-active substances, usually with a sweet taste. A special group of carbonyl compounds are oxocarboxylic acids and, in a broader sense, all carboxylic acids, which often carry a sour taste. A special group of unsaturated diketones derived from aromatic systems are quinones, which are often significant natural dyes in foods.

8.2.4.1.1 Aldehydes

Aliphatic saturated and unsaturated aldehydes

Almost all saturated aliphatic aldehydes, starting with formaldehyde (methanal) and ending with dodecanal, are important odour-active compounds. Particularly important odour-active compounds are monoterpenic aldehydes.

Typical aminocarboxylic acids, unsaturated fatty acids bound in lipids, sugars and some other food components are precursors of many important sensory-active carbonyl compounds. Amino acids produce aldehydes mainly as secondary products of alcoholic or lactic acid fermentations and during thermal processes by Strecker degradation. Formaldehyde (methanal) is formed from glycine, acetaldehyde (ethanal) from alanine; propanal and butanal arise from threonine (Figure 8.3), 2-methylpropanal from valine, 3-methylbutanal from leucine, 2-methylbutanal from isoleucine (**8-36**), 2-mercaptoethanal from cysteine and methional from methionine. Of the straight chain unsaturated aldehydes formed from lipids, particularly important are the compounds propenal (acrolein), some alk-2-enals, alk-3-enals, alka-2,4-dienals and certain other aldehydes with two, three or four double bonds. Some volatile carbonyl compounds are formed by degradation of sugars, for example formaldehyde, acetaldehyde, biacetyl and derivatives of furan-2-carbaldehyde.

R—CH=O

8-36, saturated aliphatic aldehydes
methanal (formaldehyde), R = H
ethanal (acetaldehyde), R = CH_3
propanal (propionaldehyde), R = CH_2CH_3
butanal (butyraldehyde), R = $CH_2CH_2CH_3$
2-methylpropanal (isobutyraldehyde), R = $CH(CH_3)_2$
3-methylbutanal (isovaleraldehyde), R = $CH_2CH(CH_3)_2$
2-methylbutanal, R = $CH(CH_3)CH_2CH_3$

Formaldehyde is present in various foods (such as milk, cheeses and alcoholic beverages), but only in small amounts, as it is highly reactive and enters into reaction with many food components. It is mainly in spirits that acetaldehyde is formed in large quantities, as a degradation product of sugars in the presence of microorganisms (Figure 8.2). Small amounts of acetaldehyde can likewise be found in fermented dairy products, such as yoghurt. The presence of propenal (acrolein), which results from overheated fats (triacylglycerols) or directly by dehydration of free glycerol (Figure 8.14) is a negative outcome. Small amounts of acrolein are also present in alcoholic beverages, such as beer, wine and spirits. Acrolein is an irritating aldehyde with a disagreeable odour and toxic effects. According to the International Agency for Research on Cancer (IARC), acrolein is not classifiable with respect to its carcinogenicity towards humans.

The higher homologue of acrolein is (*E*)-but-2-enal (crotonic aldehyde), which is formed by aldolisation of acetaldehyde and dehydration of the product of aldolisation, facilitated by the presence of conjugated double bonds.

A component of tomato flavour, and possibly that of other vegetables too, is the sulfur-containing aldehyde 2-methylthioethanal (**8-37**), which arises by Strecker degradation of *S*-methylcysteine

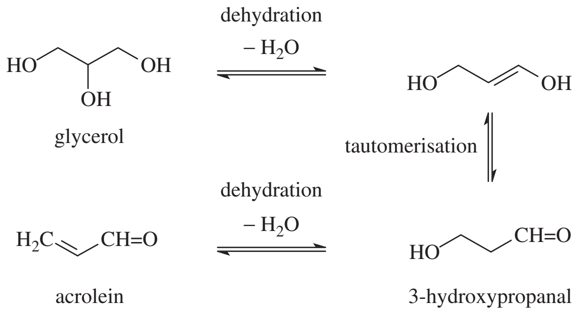
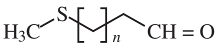


Figure 8.14 Formation of acrolein by thermal decomposition of glycerol.



8-37, methylthioalkanals
2-methylthioethanal, $n = 0$
3-methylthiopropanal (methional), $n = 1$

and its sulfoxide. The higher homologue of 2-methylthioethanal is methional (3-methylthiopropanal, **8-37**), which is formed by Strecker degradation of methionine. Methional is an important aroma component of cooked potatoes and of many other foods. However, the presence of methional in some foods has a negative effect. For example, methional (formed by riboflavin induced photooxidation) and its degradation product dimethyl disulfide give a burnt and oxidised odour to milk, as well as to wine and beer. Methional is also the key compound responsible for the defect of alcohol-free beers described as worty flavour. The other off-flavour in milk is a cardboard-like or metallic flavour, which develops in milk with a prolonged exposure to light. Compounds responsible for this off-flavour are secondary lipid oxidation products, such as hexanal, pentanal and some other substances formed by riboflavin-catalysed photooxidation of fatty acids.

Enzymatically active materials (fruits, vegetables and some fats) contain a large number of other aldehydes that are produced from essential fatty acids, mainly linoleic and linolenic acids (Table 8.9) by oxidation reactions catalysed by lipoxygenases. In some vegetables (e.g. in cucumbers), aldehydes also result from α-oxidation of fatty acids (Figure 8.15). The primary oxidation products of essential fatty acids are hydroperoxides, which break down to aldehydes and other products under the action of lyases and can be

Table 8.9 Organoleptic properties of aldehydes arising from amino acids and fatty acids.

Aldehyde	Odour	Precursor
Methanal (formaldehyde)	Pungent, sharp	Glycine
Ethanal (acetaldehyde)	Pungent, fruity, fresh	Alanine
Propanal	Pungent	Threonine, linolenic acid
2-Methylpropanal	Pungent, green	Valine
3-Methylbutanal	Green, bitter almond	Leucine
2-Methylbutanal	Green, bitter almond	Isoleucine
Methional	Boiled potatoes	Methionine
2-Phenylethanal (phenylacetaldehyde)	Floral, honey	Phenylalanine
Pentanal	Pungent	Linoleic acid
Hexanal	Tallowy, green	Linoleic acid
Heptanal	Oily, greasy	Oleic acid
Nonanal	Tallowy	Linolenic acid
(*E*)-Pent-2-enal	Oily, greasy, green	Linolenic acid
(*Z*)-Hex-3-enal	Green	Linolenic acid
(*E*)-Hex-2-enal	Oily, greasy, green	Linolenic acid
(*E*)-Hept-2-enal	Oily, greasy	Linoleic acid
(*Z*)-Oct-2-enal	Walnuts-like	Linoleic acid
(*E*)-Oct-2-enal	Oily, greasy	Linoleic acid
(*E*)-Non-2-enal	Oily, greasy	Linoleic acid
(2*E*,4*Z*)-Hepta-2,4-dienal	Oily, greasy, frying fats	Linolenic acid
(2*E*,4*E*)-Hepta-2,4-dienal	Oily, greasy	Linolenic acid
(3*Z*,6*Z*)-Nona-3,6-dienal	Cucumber-like	Linolenic acid
(2*E*,6*Z*)-Nona-2,6-dienal	Cucumber-like	Linolenic acid
(2*E*,4*Z*)-Deca-2,4-dienal	Frying fats	Linoleic acid, arachidonic acid
(2*E*,4*E*)-Deca-2,4-dienal	Frying fats	Linoleic acid
(2*E*,4*Z*,7*Z*)-Deca-2,4,7-trienal	Fish oil	Linolenic acid

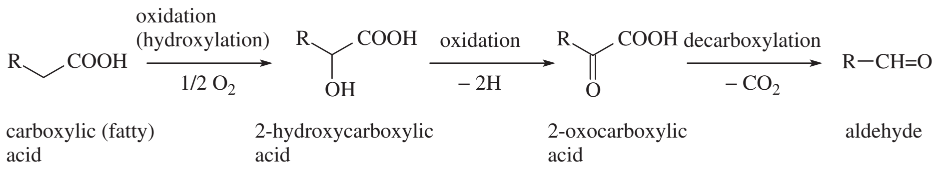


Figure 8.15 Formation of aldehydes by α-oxidation of fatty acids.

isomerised by isomerases (Figure 8.5). The same and some other hydroperoxides of linoleic and linolenic acids, their (*E*)-isomers and even oleic acid hydroperoxides (or peroxides of other unsaturated acids occurring as minor fatty acids in lipids or resulting from partial hydrogenation of unsaturated fats) are also formed by autoxidation and decompose to aldehydes and other products spontaneously (Figure 8.5 and Figure 8.6). Autoxidation of saturated fatty acids (such as palmitic and stearic acids) proceeds at higher temperatures. An overview of the main aldehydes produced from oleic, linoleic and linolenic acids is given in Table 8.10. An important indicator of the rancidity of fats is malondialdehyde. The amount of autoxidation products of unsaturated fatty acids commonly ranges in units to thousands of mg/kg. The main products of oleic acid autoxidation are octanal and nonanal, while hexanal is produced from linoleic acid (**8-38**, Figure 8.5) and (*Z*)-hex-3-enal and (2*E*,4*Z*)-hepta-2,4-dienal (**8-39**) from linolenic acid (Figure 8.6).

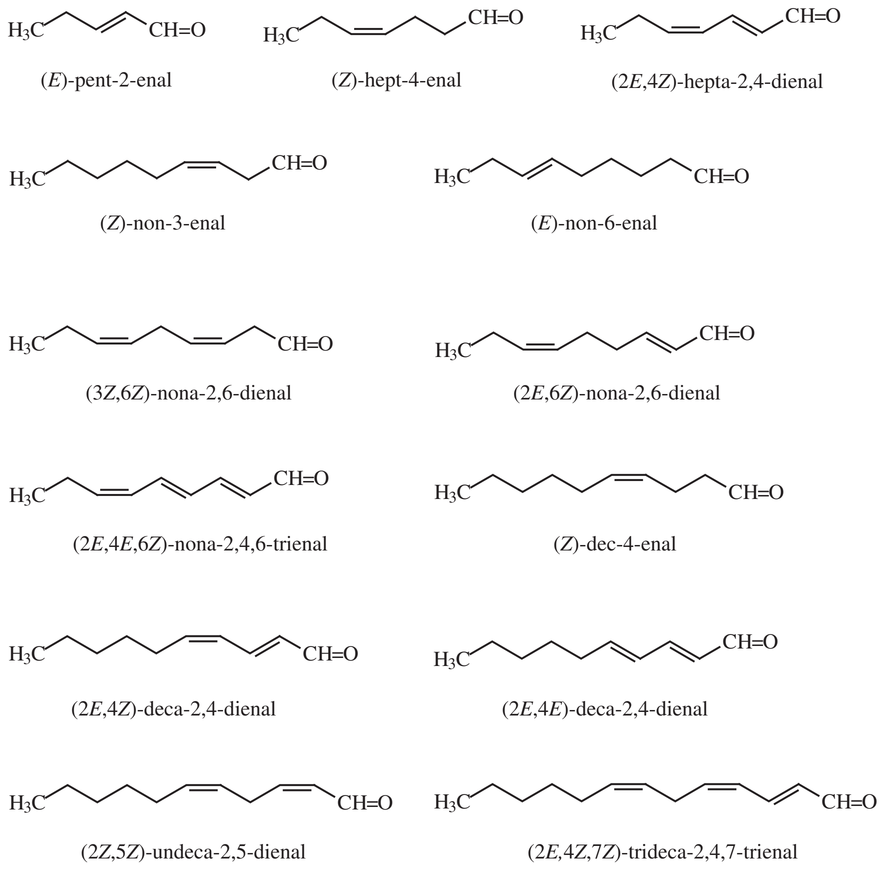
8-38, alkanals

hexanal, $n = 4$
heptanal, $n = 5$
octanal, $n = 6$
nonanal, $n = 7$

The organoleptic properties of some aldehydes produced by oxidation of fatty acids and organoleptic properties of the so-called Strecker aldehydes are listed in Table 8.9 together with their precursors. Aldehydes produced from fatty acids are often carriers of a rancid odour and taste. They are formed even from minor unsaturated fatty acids. In beef and mutton tallow and butter, for example, small amounts of (11*Z*,15*Z*)-octadeca-11,15-dienoic acid occur, autoxidation of which yields (*Z*)-hept-4-enal (**8-39**),

Table 8.10 Aldehydes arising by oxidation of unsaturated fatty acids.

Primarily formed aldehyde	Aldehyde after isomerisation	Hydroperoxy acid
(*Z*)-Undec-2-enal	(*E*)-Undec-2-enal	(*Z*)-8-Hydroperoxyoctadec-9-enoic
(*E*)-Dec-2-enal	–	(*E*)-9-Hydroperoxyoctadec-10-enoic
Nonanal	–	(*E*)-10-Hydroperoxyoctadec-8-enoic
Octanal	–	(*Z*)-11-Hydroperoxyoctadec-9-enoic
(2*Z*,5*Z*)-Undeca-2,5-dienal	(2*E*,4*E*)-Undeca-2,4-dienal	(9*Z*,12*Z*)-8-Hydroperoxyoctadeca-9,12-dienoic
(2*E*,4*Z*)-Deca-2,4-dienal	(2*E*,4*E*)-Deca-2,4-dienal	(10*E*,12*Z*)-9-Hydroperoxyoctadeca-10,12-dienoic,
(*Z*)-Non-3-enal	(2*E*)-Non-2-enal	(10*E*,12*Z*)-9-Hydroperoxyoctadeca-10,12-dienoic
(*Z*)-Oct-2-enal	(2*E*)-Oct-2-enal	(9*Z*,12*Z*)-11-Hydroperoxyoctadeca-9,12-dienoic
(*E*)-Hept-2-enal	–	(9*Z*,13*E*)-12-Hydroperoxyoctadeca-9,13-dienoic
Hexanal	–	(9*Z*,11*E*)-13-Hydroperoxyoctadeca-9,11-dienoic
Pentanal	–	(9*Z*,12*Z*)-14-Hydroperoxyoctadeca-9,12-dienoic
(2*E*,4*Z*,7*Z*)-Deca-2,4,7-trienal	–	(9*E*,12*Z*,15*Z*)-9-Hydroperoxyoctadeca-10,12,15-trienoic
(3*Z*,6*Z*)-Nona-3,6-dienal	(2*E*,6*Z*)-Nona-2,6-dienal	(8*E*,12*Z*,15*Z*)-10-Hydroperoxyoctadeca-8,12,15-trienoic
(2*Z*,5*Z*)-Octa-2,5-dienal	(2*E*,4*E*)-Octa-2,4-dienal	(9*Z*,12*Z*,15*Z*)-11-Hydroperoxyoctadeca-9,12,15-trienoic
(2*E*,4*Z*)-2,4-Heptadienal	(2*E*,4*E*)-Hepta-2,4-dienal	(9*Z*,13*E*,15*Z*)-12-Hydroperoxyoctadeca-9,13,15-trienoic
(*Z*)-Hex-3-enal	(*E*)-Hex-2-enal	(9*Z*,11*E*,15*Z*)-13-Hydroperoxyoctadeca-9,11,15-trienoic
(*Z*)-Pent-2-enal	(*E*)-Pent-2-enal	(9*Z*,12*Z*,15*Z*)-14-Hydroperoxyoctadeca-9,12,15-trienoic
Propanal	–	(9*Z*,12*Z*,14*E*)-16-Hydroperoxyoctadeca-9,12,14-trienoic



8-39, unsaturated aldehydes arising from polyenoic fatty acids

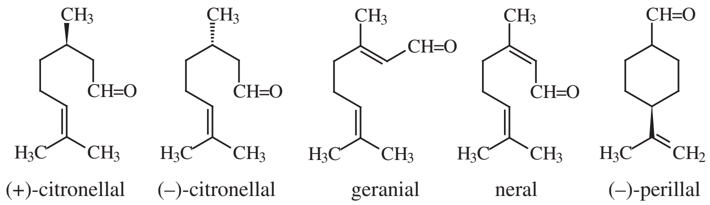
an important carrier of the rancid smell of these fats as the odour thresholds concentrations in fats are around 10 μg/kg. Important carriers of the so-called hydrogenation flavour of hydrogenated soybean oils are (*E*)-non-6-enal (**8-39**), which arises from (9*Z*,15*E*)-octadeca-9,15-dienoic acid that does not occur in natural fats, but is a product of the partial reduction of linolenic acid. An important contribution to the hydrogenation flavour of soybean oil is from (2*E*,6*E*)-octa-2,6-dienal, formed from (12*E*,16*E*)-11-hydroperoxy-12,16-octadecadienoic acid, higher alcohols and lactones.

Some aldehydes that appear from oxidised fats are desirable flavour-active compounds in low concentrations. For example, the mixture of arachidonic acid oxidation products, (*Z*)-non-3-enal, (*Z*)-dec-4-enal, (2*Z*,5*Z*)-undeca-2,5-dienal and (2*E*,4*Z*,7*Z*)-2,4,7-tridecatrienal (**8-39**) resembles the aroma of cooked chicken. Both isomers of deca-2,4-dienal (**8-39**) that arise from linoleic or arachidonic acids contribute to the smell of fried foods. The compounds, responsible for the pleasant herbal, green scent of fresh vegetables and fruits are hexanal (Figure 8.5), (*E*)-hex-2-enal (known as leaf aldehyde; Figure 8.6) and (*E*)-pent-2-enal (**8-39**), along with some alcohols The fresh cucumber scent is due to (2*E*,6*Z*)-nona-2,6-dienal and its isomer (3*Z*,6*Z*)-nona-3,6-dienal (**8-39**). The key odour-active component of oatmeal is (2*E*,4*E*,6*Z*)-nona-2,4,6-trienal (**8-39**) formed by oxidation of linolenic acid. It has a sweet, cereal-like smell with a very low odour detection threshold in air (0.000 02 ng/l). Higher aliphatic aldehydes, such as 12-methyltridecanal, have an odour resembling beef. They are formed during the thermal processing of meat by degradation of plasmalogens.

Terpenic aldehydes

Monoterpenic and some sesquiterpenic aldehydes are important flavour-active compounds with practical significance. Essential oils containing these aldehydes are used extensively in perfumery. The aliphatic aldehyde derived from (+)-citronellol is (+)-citronellal (**8-40**), which represents about 32–45% of citronella oil from the leaves and stems of grasses of the genus *Cymbopogon* (such as *C. winterianus*, Poaceae) originating from Southeast Asia (known as Javanese oil). (−)-Citronellal (**8-40**) occurs in amounts of up to 80% in the essential oil found in the subtropical Australian bush *Backhousia citriodora* (myrtle tree, Myrtaceae). Racemic citronellal (*rac*-citronellal) is a component, at levels of up to 85%, of the essential oil of plants of *Corymbia citriodora* (syn. *Eucalyptus citriodora*) from the same family of plants.

The aliphatic unsaturated aldehyde citral is one of the most frequently occurring compounds. Natural citral is always a mixture of geranial (**8-40**), also known as (*E*)-citral or citral a, and neral (**8-40**), which is known as (*Z*)-citral or citral b. The precursor of geranial and neral is geraniol. Citral occurs in the already mentioned citronella oil in concentrations of 11–13%. In the oil of the *Litsea cubeba* (Lauraceae) tree, native to Southeast Asia, citral is found in concentrations up to 75%. In much smaller amounts, citral is



8-40, monoterpenic aldehydes

a component of many other essential oils, especially oils of citrus fruits, as well as of ginger and pepper oils, and also occurs in wine and other products.

The essential oil from the leaves of the African plant *Plectranthus scutellarioides* (syn. *Perilla nankinensis* or *P. frutescens*, Lamiaceae) contains as a main component (50–60%) monocyclic (−)-perrillyl aldehyde (perillal, **8-40**), whose biosynthesis starts with (−)-limonene and proceeds via (−)-perillyl alcohol as the intermediate. Animal studies suggest that perillyl alcohol may help slow the growth of pancreatic, mammary and liver tumours.

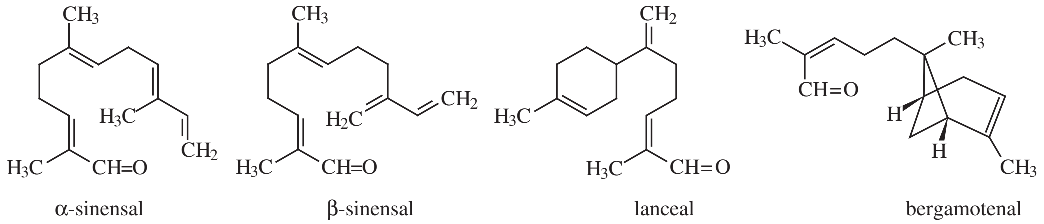
A significant acyclic sesquiterpenic aldehyde occurring in orange essential oil is α-sinensal (**8-41**), which is formed by oxidation of (3*E*,6*E*)-α-farnesene. The mandarin essential oil contains β-sinensal (**8-41**), which is produced by oxidation of (*E*)-β-farnesene (**8-7**). Some other sesquiterpenic aldehydes are minor, but still significant components of citrus oils, such as monocyclic aldehyde lanceal (**8-41**) and bicyclic aldehyde bergamotenal (**8-41**).

The alicyclic monoterpenic aldehyde safranal has a different biochemical origin, and is the main characteristic odorous component of saffron (*Crocus sativus*, Iridaceae). Safranal is classified as a degraded carotenoid (apocarotenoid) as it is produced from zeaxanthin via hydrolysis of the bitter intermediate picrocrocin. Degradation of carotenoids produces a number of other aromatic compounds (see Section 9.9.5.2). A list of the names of major terpenoid aldehydes is shown in Table 8.11.

Aromatic aldehydes

Aromatic aldehydes very often occur in essential oils and as odour-active components of different foods. Their biosynthesis is based, in principle, on transformations of phenylalanine, the key decomposition product of which is cinnamic acid (Figure 8.10).

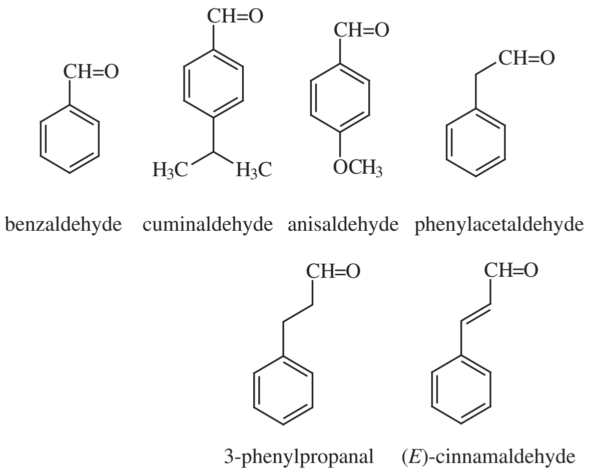
A widespread aromatic aldehyde is benzaldehyde (**8-42**), which is biosynthesised by the reduction of benzoic acid, the precursor



8-41, sesquiterpenic aldehydes

Table 8.11 Trivial and systematic names of selected terpenoid aldehydes.

Trivial name	Systematic name (IUPAC)
(+)-Citronellal	(*R*)-3,7-Dimethyloct-6-enal
(−)-Citronellal	(*S*)-3,7-Dimethyloct-6-enal
Geranial	(*E*)-3,7-Dimethylocta-2,6-dienal
Neral	(*Z*)-3,7-Dimethylocta-2,6-dienal
(−)-Perillal	(*S*)-4-Prop-1-en-2-ylcyclohex-1-ene-1-carbaldehyde
α-Sinensal	(2*E*,6*E*,9*E*)-2,6,10-Trimethyldodeca-2,6,9,11-tetraenal
β-Sinensal	(2*E*,6*E*)-2,6-Dimethyl-10-methylidenedodeca-2,6,11-trienal
Lanceal	(*Z*)-6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dienal
Bergamotenal	(1*S*,3*E*,5*S*)-4-Formyl-7-methyl-7-(4-methylpent-3-enyl)bicyclo[3.1.1]hept-3-ene



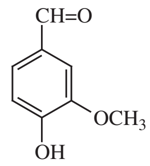
8-42, aromatic aldehydes

of which is cinnamic acid. Benzaldehyde can also be formed thermally from phenylalanine, phenylacetaldehyde and some other compounds. Benzaldehyde is present in bound form in some cyanogenic glycosides and is released by their hydrolysis (see Section 10.3.2.3.1). It is therefore a prominent component of bitter almond oil and is also present in the essential oil of cinnamon, and occurs as an important odorous component of all alcoholic beverages obtained by the fermentation of stone fruits (such as plum brandy).

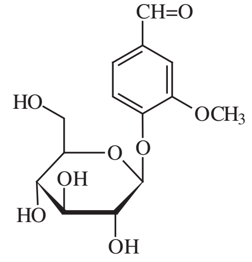
Alkyl derivatives of benzaldehyde include cuminaldehyde (4-isopropylbenzaldehyde, **8-42**), which is a component of cumin, cinnamon and basil essential oils (see Table 8.32, later). A methoxyl derivative of benzaldehyde, anisaldehyde (4-methoxybenzaldehyde, **8-42**) is a fragrance ingredient of anise, star anise, vanilla and some other essential oils. A higher homologue of benzaldehyde phenylacetaldehyde (**8-42**) is a common component of many essential oils. It has a floral aroma resembling hyacinth flowers and has therefore been used in perfumery. The cinnamon essential oil also contains its higher homologue dihydrocinnamaldehyde (3-phenylpropanal, **8-42**), which accompanies (together with cuminaldehyde) the key odorant of cinnamon, known as cinnamaldehyde (**8-42**). Cinnamon essential oil (representing about 0.5–1% by weight of cinnamon bark) contains around 90% cinnamaldehyde. The dominant isomer is the (*E*)-isomer. However, in fresh cinnamon bark, cinnamyl acetate is predominant. Cinnamaldehyde arises from this ester during fermentation by enzymatic hydrolysis and reduction of the resulting cinnamic acid by aldehyde–alcohol oxidoreductase. Without the participation of enzymes, cinnamaldehyde is produced by Strecker degradation of phenylalanine.

Other cinnamic acids also produce aldehydes, but they have no significance as odour-active substances. By analogy, coumaraldehyde is produced from 4-coumaric acid, caffeoyl aldehyde from caffeic acid, conifer aldehyde (also known as ferulyl aldehyde) from ferulic acid, 5-hydroxyconifer aldehyde from 5-hydroxyferulic acid and sinapyl aldehyde from sinapic acid. These aldehydes can be reduced to the corresponding alcohols, with which they play a role as the building units of lignin biosynthesis.

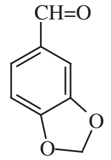
Some hydroxyaldehydes derived from benzaldehyde are also important: these can be simultaneously classified as phenols. The main characteristic component of vanilla beans (*Vanilla planifolia*, syn. V. fragrans, Orchidaceae) is vanillin (see Table 8.32, **8-43**). Vanillin is present in the green pods of orchids exclusively in the conjugated form as β-D-glucopyranoside, called glucovanillin (also known as avenin, **8-44**). The characteristic aroma of vanilla appears after fermentation (hydrolysis by β-D-glucosidase). Vanillin content in fermented pods is usually 2–2.5%. Low-quality species of vanilla contain, in addition to vanillin, the aromatic aldehyde piperonal (heliotropin, **8-45**). Vanillin can also produce many microorganisms. In biotechnological production of vanillin, different substrates are used, such as curcumin, stilbenes, eugenol and ferulic acid. Ethylvanillin (bourbonal) has an odour resembling vanillin, but slightly rougher. It does not occur in nature, but synthetic ethylvanillin is used as food flavouring, especially in the production of vanilla sugar (see Section 8.2.14.4).



8-43, vanillin



8-44, glucovanillin



8-45, piperonal

Heterocyclic aldehydes

In carbohydrate-containing foods, furan-2-carbaldehyde is often present, arising from pentoses and ascorbic acid as a dehydration product. Other common furan-derived aldehydes are 5-hydroxymethylfuran-2-carbaldehyde (from hexoses) and 5-methylfuran-2-carbaldehyde (resulting from 6-deoxyhexoses). Other heterocyclic aldehydes derived from pyrrole, thiophene, pyridine, pyrazine and other heterocyclic compounds are formed as products of the Maillard reaction.

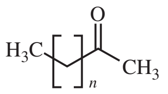
8.2.4.1.2 Ketones

As with aldehydes, various ketones can also occur as primary constituents of food raw materials and foods, or they may arise as secondary compounds in various processes. Many ketones are categorised by a characteristic odour and therefore may be either desirable or undesirable substances. An important group of ketones originating from the degradation of sugars (e.g. abhexon and sotolon) are described in the section dealing with lactones

(Section 8.2.7.1.2). A number of significant ketones arise by the degradation of carotenoids (see Section 9.9.5.2).

Aliphatic and alicyclic ketones

Foods frequently contain saturated and unsaturated aliphatic ketones with between 3 and 17 carbon atoms in the molecule. These ketones are formed by several different mechanisms. Frequently occurring aliphatic ketones are methylketones. The most common methylketone is acetone (propanone, **8-46**, $n = 0$). Acetone is present, usually in small quantities, in all biological substrates, where it arises by decarboxylation of acetoacetic (3-oxobutanoic) acid. Acetoacetic acid is formed as an intermediate during degradation of fatty acids by β-oxidation. Acetone in the skins of apples, for example, is produced from pyruvic acid via citramalic acid (Figure 8.16). The relatively large amount of acetone is generated by acetone–butanol fermentation (see Section 8.2.2.1.1). Many other saturated and unsaturated methylketones occur as odour-active components of essential oils. For example, a component of cinnamon and star anise essential oils is heptane-2-one, also known as methyl pentyl ketone (**8-46**, $n = 4$).



8-46, methylketones

Many methylketones occur in foods as secondary substances of lipid degradation during the so-called ketonic (perfume) rancidity. These methylketones have higher odour detection threshold concentrations than the corresponding aldehydes, but nevertheless play important roles as flavour-active components of some foods. They appear as desirable flavour components in cheeses with molds on the rind or throughout, such as blue cheeses (Roquefort and Gorgonzola cheeses), in the manufacture of which the mold *Penicillium roqueforti* is used. Significant methylketones (**8-46**) in blue cheeses are mainly acetone ($n = 0$), pentan-2-one ($n = 2$), heptan-2-one ($n = 4$) nonan-2-one ($n = 6$) and undecan-2-one ($n = 8$). The amount of individual compounds is between 5 and 180 mg/kg of dry matter. The last three methylketones are also used for aromatisation of cheeses.

The presence of methylketones is undesirable in some fats, such as butter, coconut and palm oil. Also undesirable are unsaturated ketones produced by oxidation of fatty acids, which are called alkyl vinyl ketones. For example, pent-1-en-3-one (**8-47**) smells of fish oil, oct-1-en-3-one (**8-47**) has a metallic taste, and (*Z*)-octa-1,5-dien-3-one (**8-47**) has an oily, greasy and metallic taste. A related ketone similarly formed by oxidation of fatty acids is (*E*)-5-methylhept-2-en-4-one (**8-47**), known as filbertone (Table 8.1), a key component of the odour of hazelnut and some chocolate products, such as nougat. Its content in raw nuts is low (about 1.4 μg/kg), but increases to 660 μg/kg in nuts roasted at 180 °C for 9 minutes, and to 1150 μg/kg in nuts roasted for 15 minutes. Hazelnuts contain a mixture of (+)-(2*E*,5*S*)- and (−)-(2*E*,5*R*)-isomers (**8-47**). The enantiomeric composition of raw hazelnuts

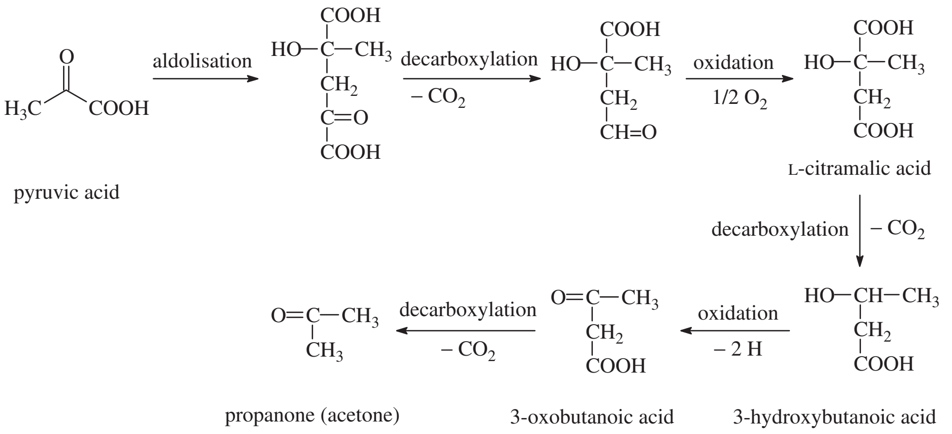
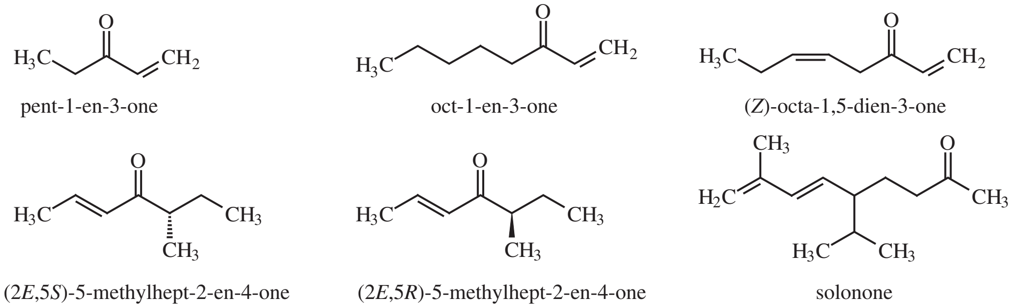


Figure 8.16 Formation of acetone from pyruvic acid.

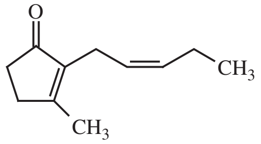


8-47, aliphatic unsaturated ketones

falls in the ratio of 80–85% (+)-(*E*,*S*) and 15–20% (−)-(*E*,*R*), whereas for roasted hazelnuts the ratio is about 71.5–72.5% (+)-(*E*,*S*) and 27.5–28.5% (−)-(*E*,*R*). The odour detection threshold for racemate (in 3% solution of sucrose) is 0.005 μg/l, and the odour recognition threshold value is 0.030 μg/l. Filbertone is also present in hazelnut oils. Oils from raw nuts contain filbertone at a level of <10 μg/kg, but its content in oils from roasted nuts is much higher (around 315 μg/kg). Identification of filbertone as a characteristic marker has been used to detect the presence of cheaper hazelnut oil in olive oil, hazelnuts in nut and cocoa spreads, and hazelnut traces in products during monitoring for hazelnut allergies (known to be one of the most common types of food allergy).

A number of unsaturated aliphatic and alicyclic ketones are formed by degradation of carotenoid pigments. For example, components of tomato flavour are unsaturated methylketones (*E*)-6-methylhept-5-en-2-one, (3*E*,5*E*)-6-methylhepta-3,5-dien-2-one (see Section 9.9.5.2.4) and other methylketones, and the flavour-active component of tobacco is (*E*)-5-isopropyl-8-methylnona-6,8-dien-2-one, known as solonone (**8-47**). Alicyclic ketones, which are the result of degradation of carotenoid pigments, include flavour-active ionones, damascones and many others (see Section 9.9.5.2.1).

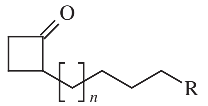
An important ketone obtained from the essential oil of jasmine flowers (*Jasminum grandiflorum*, Oleaceae) is (*Z*)-3-methyl-2-(pent-2-en-1-yl)-2-cyclopenten-1-one, also called *cis*-jasmone (**8-48**), which belongs to the group of structurally related compounds called jasmonoids (see Section 3.8.1.8.5) that are used in perfumery and cosmetics. Jasmine flowers are used to scent tea in China.



8-48, (*Z*)-jasmone

Cyclotene (2-hydroxy-3-methylcyclopent-2-en-1-one) is a secondary transformation product of sugars (see Figure 4.62) the odour of which resembles caramel. It is a characteristic aroma component of maple syrup obtained from the sugar maple tree (*Acer saccharum*, Aceraceae).

Unusual genotoxic ketones are 2-alk(en)ylcyclobutanones (**8-49**) that develop in irradiated meat and fish. The list of foods and food ingredients that may be treated with ionising radiation in the EU (Directives 1999/2/EC and 1999/3/EC) does not include meat and meat products, except for chicken meat (The Netherlands), mechanically recovered chicken meat (France) and poultry (France and the United Kingdom). 2-Alkylcyclobutanones are formed by the loss of an electron from the oxygen on the carbonyl of a free fatty acid or fatty acid bound in triacylglycerol, followed by a rearrangement to produce products having the same number of carbon atoms as the parent fatty acid. For example, 2-dodecylcyclobutanone, 2-tetradecylcyclobutanone, and (*Z*)-2-(tetradec-5′-en-1-yl)cyclobutanone have been identified in irradiated cured pork products. The amount of 2-dodecylcyclobutanone in salami irradiated at a dose of 5 kGy was 0.026 mg/kg immediately after the irradiation and 0.068 mg/kg after 60 days of storage.

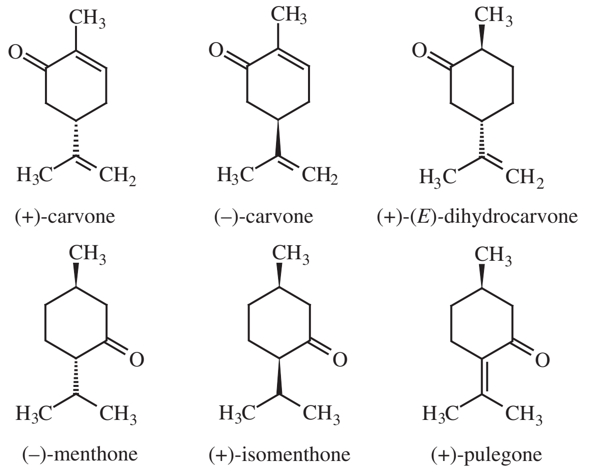


8-49, 2-alk(en)ylcyclobutanones

2-dodecylcyclobutanone, R = CH_3, *n* = 8
2-tetradecylcyclobutanone, R = CH_3, *n* = 10
(*Z*)-2-(tetradec-5′-en-1-yl)cyclobutanone, R = CH=CH $[CH_2]_7CH_3$, *n* = 1

Terpenic ketones

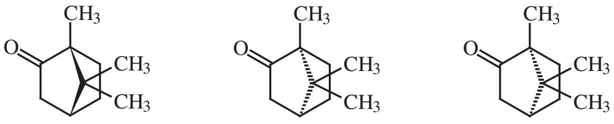
Monoterpenic ketones are frequently very important aromatic substances of many food raw materials, spices and medicinal herbs. Monocyclic ketone (+)-carvone (**8-50**) is a key component of caraway and dill oils, where it is accompanied by (+)-(*E*)-dihydrocarvone, that is (1*S*,4*S*)-dihydrocarvone (**8-50**) that also possesses caraway odour. (−)-Carvone (**8-50**) is a typical component of spearmint essential oil, where it is accompanied by (−)-(*E*)-dihydrocarvone, (1*R*,4*R*)-dihydrocarvone, which has the same odour (see Table 8.32). Isomeric (−)-menthone (**8-50**) and its precursor (+)-pulegone (**8-50**) occur in the essential oils of the European pennyroyal (*Mentha pulegium*, Lamiaceae), a traditional culinary herb and folk remedy, and other *Mentha* species (such as *M. longifolia*), as well as in marjoram essential oil. In the essential oil of mint, (−)-menthone is accompanied by (+)-isomenthone (**8-50**) and other terpenoids.



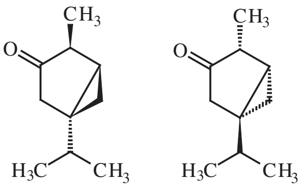
8-50, cyclic monoterpenic ketones

An example of bicyclic monoterpenic ketones is thujone, which occurs as stereoisomeric (+)-3-thujone (α-thujone, **8-51**) and (−)-3-thujone (β-thujone, **8-51**) in the sage essential oil (see Table 8.32), usually in a 1 : 2 ratio, and in other species of the genus *Artemisia* (Asteraceae). Thujone is produced from hydrocarbon sabinene by gradual oxidation and reduction. Both thujones show neurotoxic effects and therefore appear in the list of substances of Regulation (EC) No. 1334/2008, which should not be added to foods as flavourings. Their natural content is restricted to 35 mg/kg in alcoholic beverages produced from *Artemisia* species, to mg/kg in other alcoholic beverages and to 0.5 mg/ kg in non-alcoholic beverages produced from *Artemisia* species.

Bicyclic ketone camphor, formed by oxidation of borneol, is a component of cinnamon, sage and rosemary essential oils. In nature, camphor is formed by the oxidation of borneol, and usually occurs as a mixture of two isomers, (+)-camphor (**8-51**), which is more common, and (−)-camphor (**8-51**). Camphor is obtained from the camphor laurel tree wood (*Camphor officinalis*, syn. *Cinnamomum camphora*, Lauraceae) originating in Indochina, where it grows in large numbers. A further sesquiterpenic ketone is (−)-fenchone (**8-51**), which is found in many essential oils, for example in fennel essential oil.



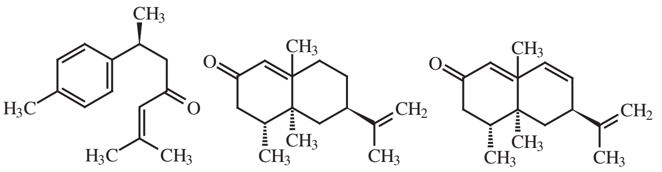
(+)-camphor (–)-camphor (–)-fenchone



α-thujone β-thujone

8-51, bicyclic monoterpenic ketones

Monocyclic sesquiterpenic ketone (+)-*ar*-turmerone (**8-52**) is the main component (25–50%) of turmeric essential oil (see Table 8.32). It is formed by oxidation of hydrocarbon (+)-*ar*-curcumene, which is present in turmeric rhizomes at a level of about 6%. Bicyclic sesquiterpenic ketone is (+)-nootkatone (**8-52**), which is an important odorous compound of grapefruits formed by oxidation of hydrocarbon (+)-valencene via alcohol (+)-β-nootkatol. Another oxidation product of valencene is (+)-8,9-didehydronootkatone (**8-52**). The trivial and systematic names of important terpenic ketones are listed in Table 8.12.

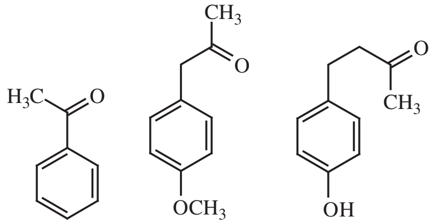


(+)-*ar*-turmerone (+)-nootkatone (+)-8,9-didehydronootkatone

8-52, sesquiterpenic ketones

Aromatic ketones

The basic substance is acetophenone, also known as phenyl methyl ketone (**8-53**), which occurs in a small amount in some essential oils. A component of fennel (see Table 8.32) and star anise essential oils is anise ketone, also known as 4-methoxyphenylacetone (**8-53**). 1-(4-Hydroxyphenyl)butan-3-one, called raspberry ketone (**8-53**), is a natural component of raspberry aroma.



acetophenone anise ketone raspberry ketone

8-53, aryl-containing ketones

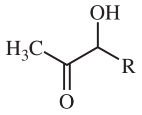
Hydroxycarbonyl and dicarbonyl compounds

Common α-hydroxycarbonyl compounds in foods include various acyloins (**8-54**), also referred to as ketols or α-hydroxyketones, which together with α-dicarbonyl compounds (**8-55**) can be

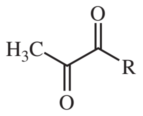
Table 8.12 Trivial and systematic names of selected terpenic ketones.

Trivial name	Systematic name (IUPAC)
(−)-Fenchone	(1*R*,4*S*)-1,3,3-Trimethylbicyclo[2.2.1]heptan-2-one
(+)-Isomenthone	(2*R*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-one
(+)-Camphor	(1*R*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one
(+)-Carvone	(*S*)-2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one
(−)-Carvone	(*R*)-2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-one
(−)-Menthone	(2*S*,5*R*)-5-Methyl-2-propan-2-ylcyclohexan-1-one
(+)-Nootkatone	(4*R*,4a*S*,6*R*)-4a,5-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-7-oxo-3-prop-1-en-2-ylnaphthalene
(+)-Pulegone	(*R*)-5-Methyl-2-propan-2-ylidencyclohexan-1-one
(+)-3-Thujone	(1*S*,4*S*,5*R*)-Thujan-3-one; [1*S*-(1α,4β,5α)]-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-3-one
(−)-3-Thujone	(1*S*,4*R*,5*R*)-Thujan-3-one; [1*S*-(1α,4α,5α,)]-4-methyl-1-propan-2-ylbicyclo[3.1.0]hexan-3-one
(+)-*ar*-Turmerone	(*S*)-2-Methyl-6-(4-methylphenyl)hept-2-en-4-one
(+)-(*E*)-Dihydrocarvone	(2*S*,5*S*)-2-methyl-5-prop-1-en-2-ylcyclohexanone
(−)-(*E*)-Dihydrocarvone	(2*R*,5*R*)-2-methyl-5-prop-1-en-2-ylcyclohexanone

considered as oxidation products of ethylene glycol (ethane-1,2-diol) and other polyols, but they are produced from other precursors. Some important hydroxycarbonyl and dicarbonyl compounds are formed as physiological secondary fermentation metabolites that can be either desirable or undesirable substances of foods.



8-54, α-hydroxyketones (acyloins)



8-55, α-dicarbonyl compounds

Important three-carbon compounds are methylglyoxal and products of its reduction, acetol and D-lactic acid aldehyde (D-lactaldehyde) (Figure 8.11). The four-carbon compounds comprise acetoin (2-hydroxybutanone) and its oxidation product biacetyl (Figure 8.12). Of the higher homologues, 3-hydroxypentan-2-one and pentane-2,3-dione are important. Acetoin, biacetyl and pentane-2,3-dione are organoleptically important compounds. The acetoin and biacetyl are flavour-active compounds that have a pleasant butter-like odour and creamy taste in diluted solutions, and are actually components of butter volatiles. For example, the biacetyl content in butter made from sour cream is around 4 mg/kg. In alcoholic beverages, such as beer and wine, biacetyl (as well as pentane-2,3-dione) is an undesirable substance as it imparts a characteristic buttery aroma to beer when present above its odour detection threshold (0.04 mg/l in air). It is an indicator of unwanted bacterial contamination. The contents of the main hydroxycarbonyl and dicarbonyl compounds in wine are given in Table 8.13. Red wines tend to have slightly higher levels of acetoin and biacetyl than white wines.

In addition to the already mentioned acyloins, food products contain numerous other odorous α-hydroxyketones. For example, 3-hydroxypentan-2-one has ben identified in cheeses, durian, wines, sherry, asparagus, honey, tea, butter and soy sauce, 2-hydroxypentan-3-one in cheeses, durian, coffee, wine, sherry honey, butter and soy sauce, 2-hydroxyhexan-3-one in wine, 4-hydroxyhexan-3-one in durian and tea, 3-hydroxy-5-methylhexan-2-one in cheeses, 3-hydroxyoctan-2-one in beef and heated mutton fat, 5-hydroxyoctan-4-one in cocoa and 3-hydroxy-4-phenylbutan-2-one in wine, sherry and honey.

Table 8.13 Content of main hydroxycarbonyl and dicarbonyl compounds in wines.

Compound	Content (mg/l)
Propane-1,2-dione (methylglyoxal)	0.06–0.42
3-Hydroxybutan-2-one (acetoin)	0.7–53
Butane-2,3-dione (biacetyl)	0.1–7.5
3-Hydroxypentan-2-one	0.5–2.8
Pentane-2,3-dione	7.0–18

Some α-hydroxycarbonyl and α-dicarbonyl compounds, such as glycolaldehyde, 1-hydroxypropanone (hydroxyacetone or acetol), glyoxal (oxalic acid dialdehyde), methylglyoxal (pyruvic acid aldehyde), 3-hydroxybutan-2-one (acetoin) and many others are important products of carbohydrate degradation. These compounds are generally not characterised by distinct organoleptic properties, but are precursors to many important compounds, especially heterocyclic volatiles formed in the Maillard reaction. The 1,2-dicarbonyl compound glyoxal, 1,3-dicarbonyl compound malondialdehyde and various ketols are similarly formed as products of fatty acid oxidation.

8.2.4.2 Properties and reactions

Aliphatic aldehydes with between 1 and 7 carbon atoms in the molecule generally have a sharp, pungent and sometimes rancid aroma, while aldehydes with 8–14 carbon atoms (including monoterpenic aldehydes) are generally characterised by a pleasant aroma. Higher aldehydes are usually odourless.

Symmetric and asymmetric aliphatic ketones of low molecular weight have a pleasant smell. Higher ketones, especially, methyl ketones, have a characteristic smell, which is sometimes desirable and sometimes not. Flavour-active substances in the essential oils of different spices are various monoterpenic ketones. Aliphatic ketones of medium chain length and terpenic ketones are used as flavouring substances in foods and cosmetic products. In the food flavouring industry, some α-hydroxycarbonyl compounds, such as acetoin and some α-dicarbonyl compounds (e.g. biacetyl) are usually mixed with other ingredients to produce a butter flavour or other flavours in a variety of food products, including butter made from sweet cream, margarine and popcorn. Pentane-2,3-dione does not have a significant impact on the flavour of foods.

The most important reactions of carbonyl compounds are addition, oxidation and reduction taking place on the carbonyl group. Owing to the presence of free electron pairs on the carbonyl group oxygen, carbonyl compounds are weak bases, and in acidic media may exist in protonated forms (as conjugated acids; Figure 8.17). Bases split the acidic hydrogen on the carbons adjacent to the carbonyl group to form reactive carbanions (Figure 8.18). Cleavage of hydrogen from the carbon in position α to the carbonyl group and its addition to the carbanion oxygen leads to an equilibrium between the oxo form and the enol form. This isomerisation reaction is called enolisation (Figure 8.19). Enolisation is catalysed by both acids and bases, thus it is an acid–base catalysed reaction. Under normal conditions, the equilibrium in simple aldehydes

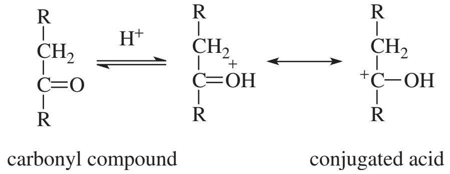


Figure 8.17 Formation of conjugated acids from carbonyl compounds in acidic media.

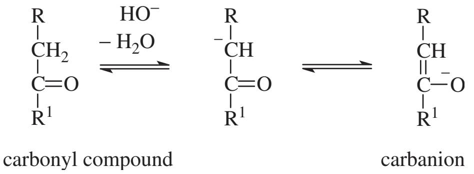


Figure 8.18 Formation of carbanions from carbonyl compounds in basic media.

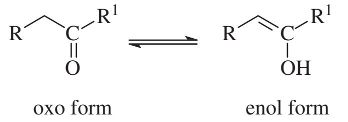


Figure 8.19 Enolisation of carbonyl compounds.

and ketones is almost completely shifted in favour of carbonyl compounds. However, if the methylene group hydrogens are activated, as is the case with malondialdehyde, the proton splits easily and the dominating form of such a carbonyl compound is then the thermodynamically stable enol form.

8.2.4.2.1 Addition reactions

Carbon of the carbonyl group reacts readily with nucleophilic reagents. The first step is an addition of a nucleophile on the carbon atom of the polarised carbonyl group double bond, which is followed by reaction with an electrophile, usually with a proton (Figure 8.20). The most important addition reactions of carbonyl compounds are reactions with water, alcohols, sulfan, thiols, ammonia, amines and aminocarboxylic acids. Reactions are generally acid–base catalysed, so are influenced to a great extent by pH. Addition of acid leads to the formation of a more reactive conjugated acid, but also reduces the concentration of the nucleophile. An addition of hydroxides has the opposite effect. The ease of addition primarily affects the inductive effect of substituents. Alkyl and aryl substituents have a positive inductive effect (+I), and the reactivity of the carbonyl compounds, therefore, decreases in the following order: formaldehyde > aromatic aldehydes > higher aliphatic aldehydes, unsaturated aldehydes > methyl ketones > alkyl aryl ketones > dialkyl ketones. The presence of electronegative functional groups with negative inductive effect (–I) in the aliphatic substituent in the position adjacent to the carbonyl group (such as another carbonyl group or hydroxy group) results in correspondingly higher reactivity of the carbonyl group. Reactivity then declines in the order: α-dicarbonyl compounds (such as glycosuloses) and α-hydroxycarbonyl compounds (such as current aldoses and ketoses). If the carbonyl group is conjugated with a double bond (e.g. in alk-2-enals), the 1,4-addition of nucleophilic reagents may occur (Figure 8.21).

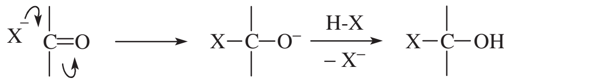
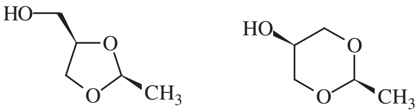


Figure 8.20 Nucleophilic addition to carbonyl compounds (X = OH, SH, NH_2, NH–R).

Reactions with water and alcohols

The addition of water to the carbonyl group in aqueous solutions can lead to the formation of hydrates. The reactions and stability of hydrates depend primarily on the inductive effect of substituents. Hydration of formaldehyde readily yields methylene glycol (methanediol), which polymerises to linear oligomers and also to polymers known as paraformaldehyde. Hydrates of α-dicarbonyl and α-hydroxycarbonyl compounds spontaneously dimerise into various cyclic 1,4-dioxanes (see Figure 4.62). These hydrates are intermediates of other α-dicarbonyl and α-hydroxycarbonyl compounds in the oxidation–reduction reactions and precursors of carboxylic acids. Dialkyl ketones do not form hydrates.

Adducts resulting from reactions of carbonyl compounds with alcohols (also with sugars) are called hemiacetals. This reaction (1,2-addition) is catalysed by acids, and primary alcohols are more reactive than secondary alcohols. Splitting a water molecule in acidic media yields a conjugate acid that reacts with another molecule of alcohol with the formation of **acetals**, products of ketones with alcohols that were formerly known as ketals (Figure 8.22). The formation of acetals has considerable importance in the development of flavour-active compounds in alcoholic beverages. In the case of alk-2-enals (such as acrolein), the reaction proceeds in a manner identical with alkene electrophilic addition and, in addition to the 1,2-addition, 1,4-addition is also possible (Figure 8.23). Reactions of glycols with carbonyl compounds yield cyclic acetals, known as that 1,3-dioxolanes (Figure 8.24). The condensation reaction between glycerol and acetaldehyde under acid conditions (at the pH of wine) leads analogously to the formation of four isomers: (*Z*)- and (*E*)-5-hydroxy-2-methyl-1,3-dioxane and (*Z*)- and (*E*)-4-hydroxymethyl-2-methyl-1,3-dioxolane (**8-56**). It is expected that these acetals contribute to the aroma of old sherry and Port wines.



(*Z*)-4-hydroxymethyl-2-methyl-1,3-dioxolane (*Z*)-5-hydroxy-2-methyl-1,3-dioxane

8-56, reaction products of glycerol with acetaldehyde

Acetals are stable in neutral and alkaline solutions. Those derived from aldehydes are more stable than acetals derived from ketones

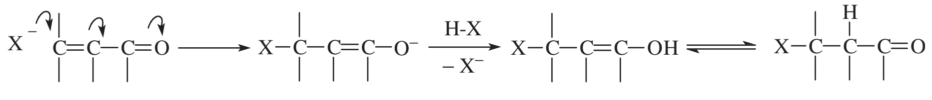


Figure 8.21 1,4-Addition to carbonyl compound double bond.

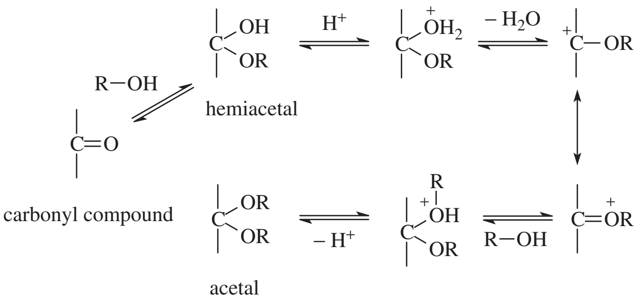


Figure 8.22 Addition of alcohols and formation of acetals.

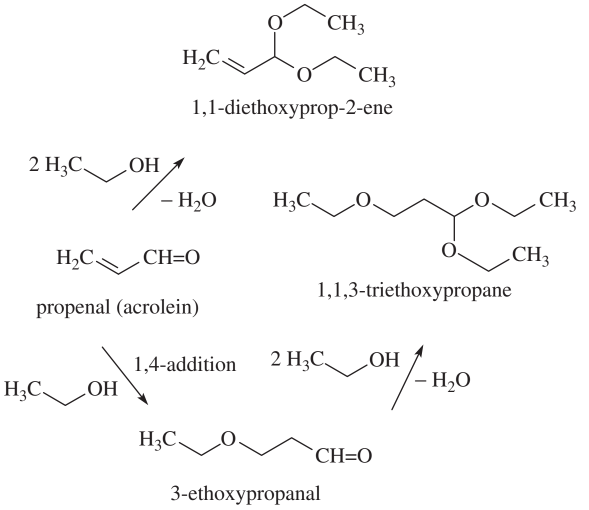


Figure 8.23 Reactions of acrolein with ethanol.

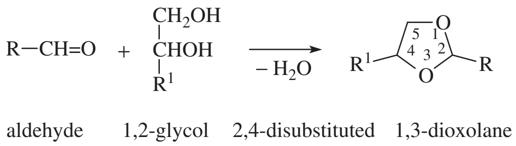


Figure 8.24 Formation of cyclic acetals from aldehydes and 1,2-glycols.

and cyclic acetals are more stable than aliphatic acetals. In acidic solutions, they are hydrolysed to the parent compounds.

Reactions with sulfan and thiols

Similarly to water and alcohols, carbonyl compounds react with sulfan (hydrogen sulfide) and thiols. Addition of sulfan gives unstable α-hydroxythiols as intermediates (Figure 8.25). Mercapto alcohols easily dehydrate to thioaldehydes or thioketones, form cyclic compounds (mostly trimers, which are termed trithioaldehydes and trithioketones) or linear polymers. The reaction is of great importance, for example, in the formation of many aromatic compounds arising during the thermal processing of meat. An example is the reaction of acetaldehyde with sulfan (Figure 8.26).

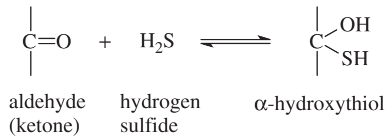


Figure 8.25 Reaction of carbonyl compounds with hydrogen sulfide.

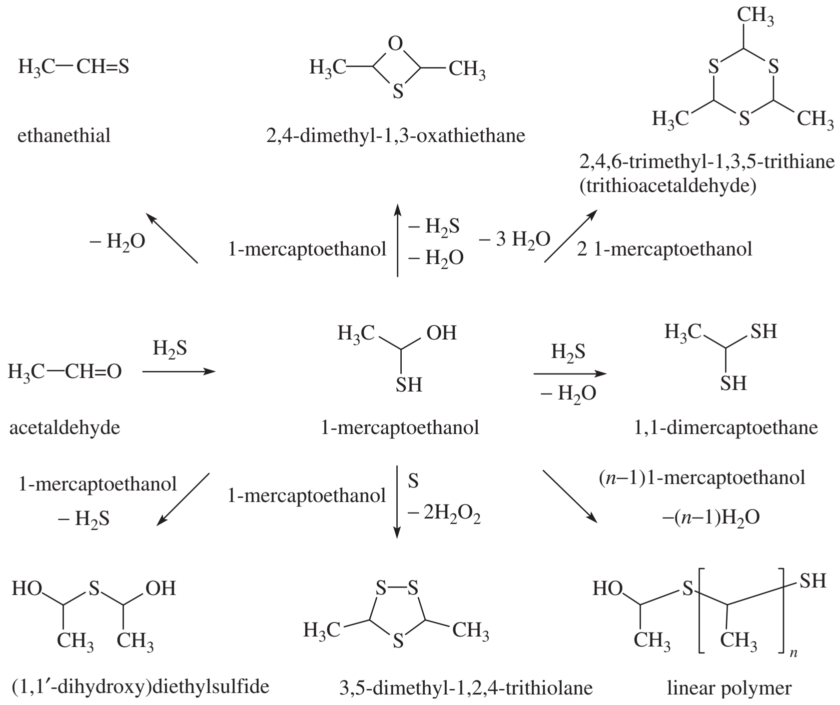


Figure 8.26 Reactions of acetaldehyde with hydrogen sulfide.

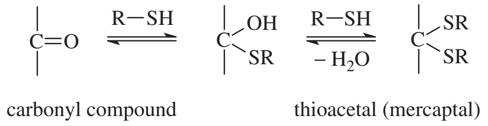


Figure 8.27 Reactions of carbonyl compounds with thiols.

Thiols react with aldehydes and ketones to form thioacetals (mercaptals). The reaction equilibrium is, in comparison with the reaction of alcohols, shifted more in favour of products that are mainly involved as flavour-active components of meat, vegetables and mushrooms (Figure 8.27). The reaction intermediates (semithioacetals) give rise to a number of other compounds.

Reactions with ammonia and amines

Addition products of aldehydes and ketones with ammonia and amines (hemiaminals) are unstable compounds that are then stabilised by a number of subsequent reactions (Figure 8.28). Dehydration of addition products yields imines. Other possible products are enamines, amines containing a double bond linkage –CH=CH–NH– (Figure 8.29), cyclic trimers (symmetrical 1,3,5-triazines) and various coloured polymers, generally of unknown structures. Reaction of the addition products with another amine molecule gives rise to aminals, reaction with another molecule of the carbonyl compounds yields α,α′-dihydroxyamines. For example, in addition to 1,3,5-triazines, it is mostly polymers that are formed from aliphatic aldehydes and ammonia or amines. The reaction of formaldehyde with ammonia yields hexamethylenetetramine,

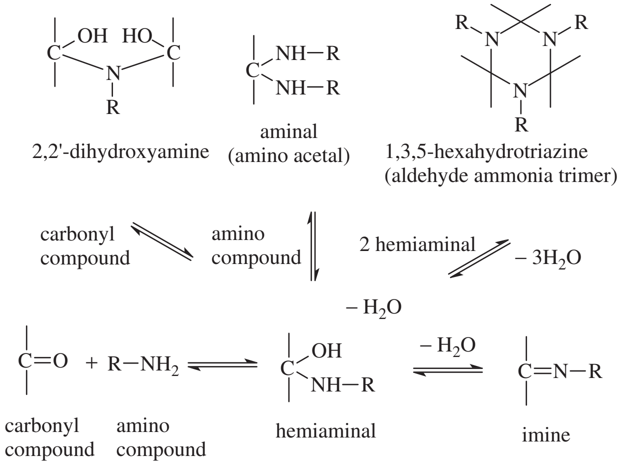


Figure 8.28 Reactions of carbonyl compounds with ammonia (R = H) and amines.

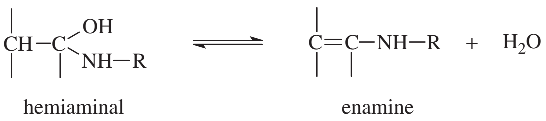
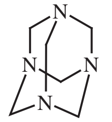


Figure 8.29 Formation of enamines.

known as urotropine (**8-57**). If cyclisation of the intermediate is possible, stable heterocyclic compounds may be formed, such as those produced in reactions of aldehydes with some aminocarboxylic acids. However, the main reaction products are polymeric brown pigments called melanoidins (see Section 4.7.5.7).



8-57, hexamethylenetetramine

Aldol condensation

Aldol condensation or aldolisation is a reaction of aldehydes and ketones, which have at least one hydrogen on the α-carbon (in position C-2 to the carbonyl group). The nucleophile in aldolisation is an anion of the carbonyl compound. It reacts with the carbonyl group of the second aldehyde or ketone molecule to form α-hydroxycarbonyl compounds, called aldols. Up to this point the reaction is an aldol reaction. The following reaction step is an elimination reaction of water. The aldol produced dehydrates at elevated temperatures, in the presence of a strong base or in acidic media, yielding an α,β-unsaturated aldehyde with conjugated double bonds. Saturated aldehydes produce unsaturated aldehydes, alk-2-enals (Figure 8.30). For example, aldolisation of acetaldehyde and dehydration of the product provides both enantiomers of but-2-enal, which is also known as crotonic acid aldehyde or crotonaldehyde (Figure 8.31). Formaldehyde can react with other aldehydes or ketones having at least one hydrogen atom on the α-carbon to the carbonyl group. High reactivity of formaldehyde causes aldolisation of other molecules of formaldehyde, the number of which corresponds to the number of α-hydrogen atoms available in the reaction partner. For example, acetaldehyde reacts with formaldehyde with formation of mono-, di- and trimethylolacetaldehyde, which yields pentaerythritol by a Cannizzaro reaction (Figure 8.32).

The aldolisation of acetone in alkaline media yields diacetone alcohol, which, on heating in the presence of traces of acids,

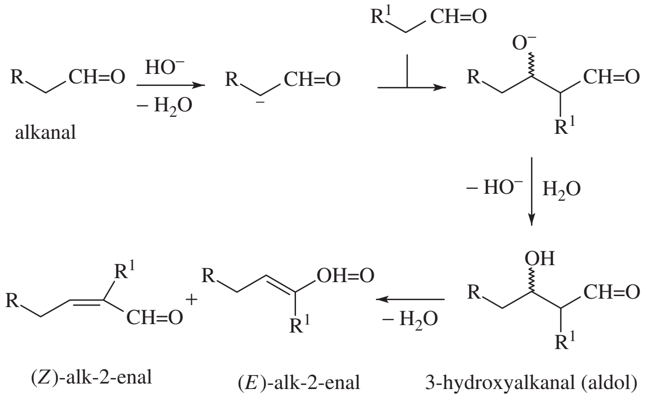


Figure 8.30 Aldolisation of carbonyl compounds.

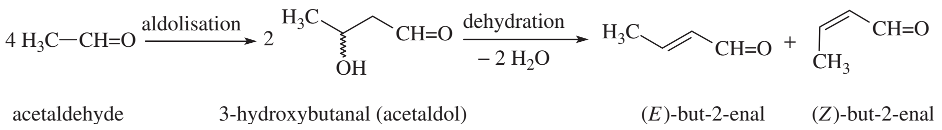


Figure 8.31 Aldolisation of acetaldehyde.

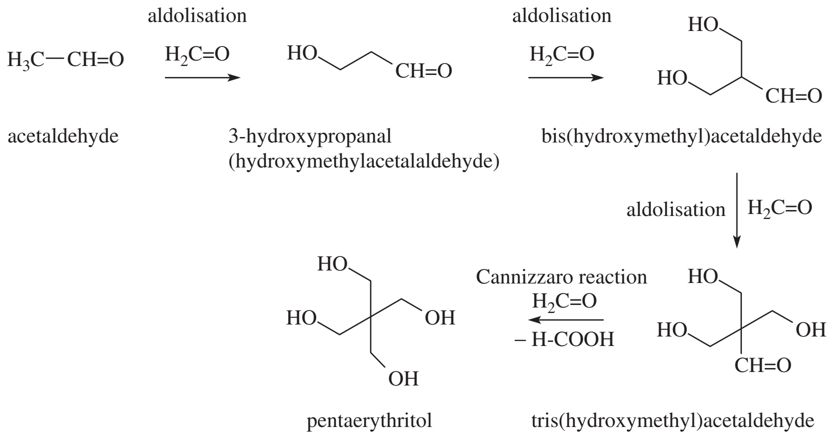


Figure 8.32 Reactions of formaldehyde with acetaldehyde.

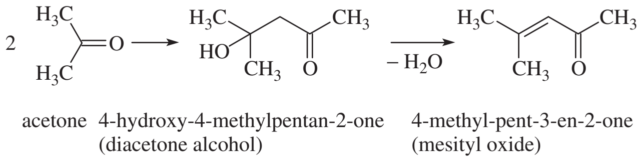


Figure 8.33 Aldolisation of acetone.

produces mesityl oxide (4-methylpent-3-en-2-one) with the aroma resembling peppermint (Figure 8.33). Mesityl oxide is a precursor of other aromatic compounds.

8.2.4.2.2 Oxidations and reductions

The carbonyl group of aldehydes, in contrast to the carbonyl group of ketones, is readily oxidised by oxygen to a carboxyl group. The primary product of autoxidation is a peroxyacid that oxidises another molecule of aldehyde to form two molecules of carboxylic acids (Figure 8.34). The autoxidation of formaldehyde gives rise to formic acid, acetaldehyde yields acetic acid, benzaldehyde gives benzoic acid and furan-2-carbaldehyde gives 2-furancarboxylic acid, also known as furoic or pyromucic acid. The oxidation of a ketone carbonyl group in food does not occur. The hydrocarbon chains of aldehydes and ketones are also subject to autoxidation, as are the hydrocarbon chains of fatty acids.

Reduction of aldehydes generates primary alcohols; ketones are formed by reducing secondary alcohols. This is usually an enzymatic reaction. For example, benzyl alcohol arises from benzaldehyde (see Section 8.2.2.1.2).

Cannizzaro reaction

A specific case of an oxidation–reduction reaction is the Cannizzaro reaction, in which one molecule of aldehyde is oxidised with

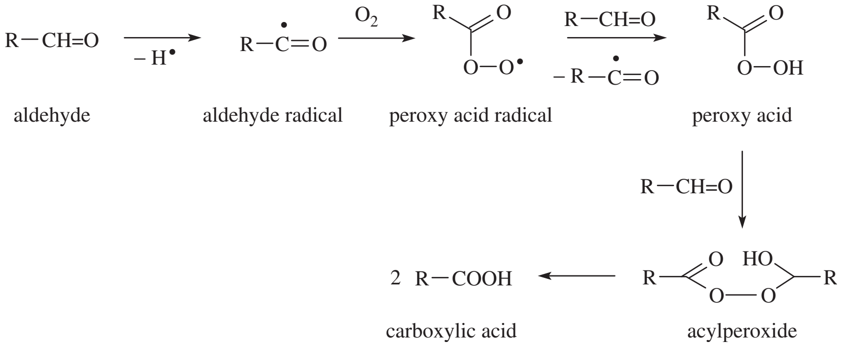


Figure 8.34 Oxidation of aldehydes to carboxylic acids.

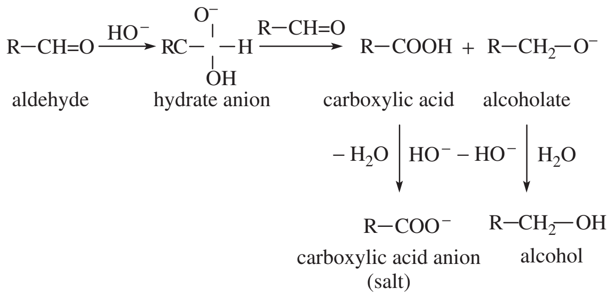


Figure 8.35 Cannizzaro reaction of aldehydes.

reduction of the second molecule. A Cannizzaro reaction occurs in aldehydes that do not have a hydrogen atom in position α to the carbonyl group. The reaction products are mixtures of primary alcohols and carboxylic acids. A Cannizzaro reaction is catalysed by hydroxyl ions; the key step is the transfer of the hydride from the hydride anion to the second aldehyde molecule (Figure 8.35).

For example, Cannizzaro reaction of formaldehyde gives methanol and formic acid. Methanol reacts with another molecule of formaldehyde (its hydrate) to form acetal 1,1-dimethoxymethane, also known as 2,4-dioxapentane. The reaction of methanol with formaldehyde hydrate oligomers yields, by analogy, higher dioxaalkanes. The Cannizzaro reaction of benzaldehyde also proceeds easily (especially in the presence of heavy metal traces), yielding benzyl alcohol and benzoic acid. Benzoic acid is otherwise also formed by autoxidation of benzaldehyde. The Cannizzaro reaction of furan-2-carbaldehyde yields furfuryl alcohol and 2-furancarboxylic acid.

In the presence of formaldehyde, a Cannizzaro reaction even occurs with aldehydes, which do not have hydrogen atoms on the carbon in position α to the carbonyl group. Formaldehyde is then oxidised to formic acid, and the aldehyde is reduced to the corresponding alcohol (Figure 8.36). Reaction of formaldehyde with aldoses produces small amounts of formic acid and the corresponding alditol.

In the case of 2-oxoaldehydes, such as glyoxal and methylglyoxal, the Cannizzaro reaction proceeds in the same way as the intramolecular reaction, and the product is 2-hydroxycarboxylic acid. The aldehyde group of oxo aldehyde is oxidised to a carboxylic group with the simultaneous reduction of the oxo group to a hydroxyl group (Figure 8.37). For example, glyoxal yields glycolic acid and methylglyoxal yields lactic acid (racemate).

8.2.5 Acetals

8.2.5.1 Classification, structure, terminology and occurrence

Acetals occur wherever aldehydes and ketones are present with an excess of alcohol. Relatively large amounts of acetals are therefore found in alcoholic beverages, particularly spirits with higher ethanol content. However, even there the proportion of aldehydes bound as acetals does not usually exceed 15–30% of the total aldehyde content.

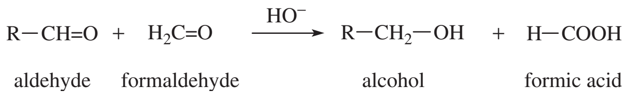


Figure 8.36 Cannizzaro reaction of aldehydes and formaldehyde.

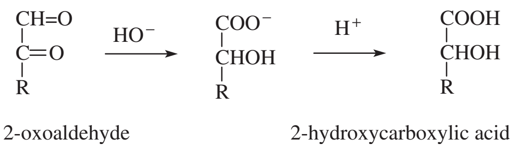
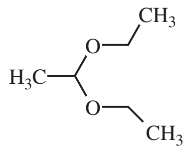


Figure 8.37 Cannizzaro reaction of α-dicarbonyl compounds.

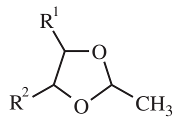
Acetaldehyde is more reactive than higher alkanals, alkenals and aromatic aldehydes, and is present in alcoholic beverages in a relatively high concentration. Therefore, the most common substance found in alcoholic beverages is so-called diethylacetal (1,1-diethoxyethane) resulting from the reaction of acetaldehyde with ethanol (**8-58**). It is characterised by a sharp fruity aroma and a flavour reminiscent of nuts. The content, depending on the time of ripening of the whiskies and brandies, varies from about 4 to 60 mg/l. Acetals derived from formaldehyde and ethanol and acetals derived from higher aldehydes are present in lower amounts, as well as acetals arising from fusel oil alcohols.



8-58, 1,1-diethoxyethane

Reaction of the unsaturated aldehyde acrolein with ethanol leads either to 1,1-diethoxyprop-2-ene or to 3-ethoxypropanal. Both compounds can react with another ethanol molecule to give the final product, 1,1,3-triethoxypropane (Figure 8.23). All three compounds are found in alcoholic beverages.

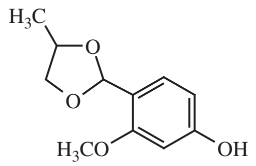
1,3-Dioxolanes (**8-59**) are cyclic acetals resulting from the condensation of carbonyl compounds with glycols in acidic media. They are common in alcoholic beverages. Brandies and other spirits contain 4-hydroxymethyl-2-methyl-1,3-dioxolane ($R^1 = CH_2OH$, $R^2 = H$), which forms from acetaldehyde and glycerol, 2,4-dimethyl-1,3-dioxolane ($R^1 = CH_3$, $R^2 = H$) is a product of acetaldehyde and propane-1,2-diol (propylene glycol) condensation (propane-1,2-diol is a product of methylglyoxal



8-59, 1,3-dioxolanes

reduction), 2,4,5-trimethyl-1,3-dioxolane ($R^1 = R^2 = CH_3$) results from acetaldehyde and butane-2,3-diol.

The most commonly occurring cyclic acetals of commercial aromas are 1,3-dioxolane derivatives that are derived from propane-1,2-diol used as a solvent. In vanilla flavours, for example, a considerable amount of 2-(4-hydroxy-2-methoxyphenyl)-4-methyl-1,3-dioxolane (**8-60**) occurs, which is formed in the reaction of propane-1,2-diol with vanillin.



8-60, 2-(4-hydroxy-2-methoxyphenyl)-4-methyl-1,3-dioxolane

8.2.5.2 Properties and reactions

Acetals, especially those compounds derived from lower alcohols, generally have a pleasant smell similar to the original carbonyl compounds, but weaker and softer. Those derived from higher alcohols usually have pleasant smells, associated with aging of alcoholic beverages. Acetals are more stable than the corresponding aldehydes and ketones, especially in alkaline media and during oxidation. Therefore, they are often used as flavourings for soaps. Under acidic conditions, acetals are easily hydrolysed to the parent compounds.

8.2.6 Carboxylic acids

Carboxylic acids are particularly important components of foods of plant origin. They influence the course of enzymatic and chemical reactions, the microbiological stability of foods during storage and processing and the organoleptic and technological properties. In foods the predominant compounds that can be found are aliphatic, alicyclic, aromatic and heterocyclic carboxylic acids that contain one carboxyl group (monocarboxylic acids) or more carboxyl groups (polycarboxylic acids) in the molecule. Many carboxylic acids also contain other functional groups such as hydroxyl groups (hydroxycarboxylic acids) and carbonyl groups (oxocarboxylic acids), amino groups (aminocarboxylic acids), mercapto groups (mercaptocarboxylic acids); certain halogen-containing carboxylic acids, the molecule of which contains, for example, one or more chlorine atoms, are food contaminants .

It is mainly the lower carboxylic acids and some aromatic carboxylic acids that are active as odour- or taste-active compounds. Taste-active substances are predominantly polyhydric carboxylic acids and some aliphatic carboxylic acids such as acetic and lactic acids, which are major carriers of the sour taste in food raw materials and foods. Short chain fatty acids also have some importance as flavour-active substances (C_4 and C_6) and medium chain fatty acids (C_8–C_{12}). A number of carboxylic acids can become precursors of important flavour-active derivatives, such as, for example, esters and lactones.

8.2.6.1 Classification structure, terminology and occurrence

8.2.6.1.1 Aliphatic monocarboxylic acids

Important odour-active acids are mainly saturated monocarboxylic acids containing 1–10 carbon atoms in the molecule (**8-61**), which in apart from the carboxyl group do not contain other functional groups. Acids with four or more carbon atoms in the molecule are classified as fatty acids (see Section 3.3.1.1).

R—COOH

8-61, lower carboxylic acids

formic(methanoic), R = H
acetic(ethanoic), R = CH_3
propionic(propanoic), R = CH_2CH_3
butyric(butanoic), R = $[CH_2]_2CH_3$
isobutyric(2-methylpropanoic), R = $CH_2CH(CH_3)2$
valeric(pentanoic), R = $[CH_2]_3CH_3$
isovaleric(3-methylbutanoic), R=$CH_2CH_2CH(CH_3)2$
caproic(hexanoic), R = $[CH_2]_4CH_3$
caprylic (octanoic), R = $[CH_2]_6CH_3$
capric(decanoic), R = $[CH_2]_8CH_3$

The basic member of the homologous series of these acids is formic acid. It occurs as a free compound and is esterified mainly in vegetables, fruits and also in alcoholic beverages, where it arises (in addition to ethanol and acetic acid) as a byproduct of the anaerobic fermentation of sugars by some microorganisms. The precursor of formic acid is pyruvic acid that reacts with HS-CoA with the formation of acetyl-CoA and formic acid, which is decomposed into carbon dioxide and hydrogen. This reaction is catalysed by pyruvate formate-lyase. Formic acid is also produced in the seeds and leaves of plants by α-oxidation of fatty acids. The product of oxidation is α-hydroxycarboxylic acid which is transformed into formic acid and carbon dioxide. The final product is a fatty acid containing one less carbon atom.

Sugars (hexoses) yield formic acid as a product of the Cannizzaro reaction of formaldehyde and as one of the final products of the dehydration of sugars in acidic media and their degradation in neutral and alkaline media. At high concentrations (in quantities of up to 2%), formic acid therefore occurs in acid protein hydrolysates, where it is formed by 5-hydroxymethyl-2-furancarbaldehyde degradation. In rancid fats, formic acid arises by oxidative decomposition of aldehydes. Formic acid is sometimes used as a preservative.

Acetic acid (**8-61**) is the most common monocarboxylic acid occurring in foods. It is a typical component of fruits (as the free acid and its esters) and foods produced by fermentation processes. Acetic acid is produced as a degradation product of sugars in the Maillard reaction and in thermal processes. For food purposes, acetic acid is produced from ethanol or fruit wines as vinegar by microbial oxidation of ethanol via acetaldehyde using aerobic bacteria of the genus *Acetobacter* (Figure 8.38). In the manufacture of vinegar, the bacterium *Acetobacter aceti* is traditionally used. The fermented ethanol (including wines) has a resulting acetic acid concentration of about 5%, with a pH of about 2.4. Any type of vinegar can be distilled to produce a colourless solution that contains about 8% acetic acid.

The higher homologues of acetic acid, propionic, butyric, isobutyric, valeric, isovaleric, caproic acid and some other acids, are formed by different mechanisms. Butyric acid, caproic, caprylic

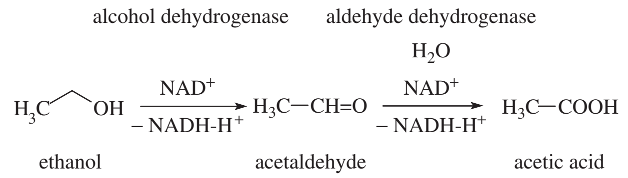


Figure 8.38 Oxidation of ethanol and formation of acetic acid.

and capric acids occur, along with other acids (such as isoacids, anteisoacids and acids with an odd number of carbon atoms), in relatively high quantities in milk fat, in the form of triacylglycerols. Their biosynthesis is primarily based on acetyl-CoA (see Section 3.2). Propionic acid is biosynthesised if the parent compound is propionyl-CoA instead of acetyl-CoA. Numerous other acyl-CoAs are intermediates in the biosynthesis of amino acids formed by elongation of 2-oxoacids under the catalytic activity of acyl-CoA synthetases. Examples of these acyl-CoAs are butanoyl, 2-methylpropanoyl-, pentanoyl-, 3-methylbutanoyl-, 4-methylpentanoyl- and hexanoyl-CoA. The corresponding 2-oxoacids are formed as amino acid catabolic products by transamination catalysed by transaminases, or by oxidative deamination catalysed by oxidases (see Section 2.5.1.3.2). Their decarboxylation catalysed by decarboxylases yields aldehydes that are finally oxidised to carboxylic acids by aldehyde dehydrogenases. For example, valine is the precursor of isobutyric acid, isovaleric acid arises from leucine, 2-methylbutyric acid from isoleucine and propionic acid from threonine. These acids are the byproducts of fermentation processes (Table 8.14).

Carbohydrates are also precursors of carboxylic acids. Bacteria of the genera *Clostridium*, *Butyribacterium* and *Butyrivibrio* ferment sugars primarily to acetic and butyric acids. Bacteria of the genus *Propionibacterium* (*P. freudenreichii* subsp. *shermanii*) ferment lactose to lactic acid, which is reduced to propionic acid, an important acid of the most famous Swiss cheese, Emmental (Figure 8.39). The first step of this biosynthesis is the reaction of pyruvic acid with (*S*)-malonyl-CoA catalysed by methylmalonyl-CoA carboxyl transferase, which yields propionyl-CoA and 2-oxaloacetic (2-oxosuccinic) acid. 2-Oxaloacetic acid is reduced to (*S*)-2-hydroxybutanedioic (L-malic) acid by malate dehydrogenase, dehydrated to (*E*)-but-2-enedioic (fumaric) acid by fumarase, which is reduced to succinic acid by succinate dehydrogenase. In the reaction catalysed by propionyl-CoA:succinate-CoA transferase, propionyl-CoA is used in the next step as a donor of coenzyme A, which creates succinyl-CoA and propionic acid as the final reaction product. Succinyl-CoA is then transformed into (*R*)-methylmalononyl-CoA by methylmalonyl-CoA with mutase, isomerisation of which by methylmalonyl-CoA epimerase yields the starting compound (*S*)-methylmalonyl-CoA.

Table 8.14 Lower carboxylic acids contents in beer.

Acid	Content (mg/l)	Acid	Content (mg/l)
Acetic	12–155	Isovaleric	0.4–3.4
Butyric	0.6–2.6	Caproic	0.9–22.8
Isobutyric	0.3–3.3	Caprylic	1.8–15.4
Valeric	0.1–0.2	Capric	0.1–5.2

An important lower unsaturated carboxylic acid is (*E*)-but-2-enoic (crotonic) acid (**8-62**). This acid occurs in small quantities, together with other unsaturated acids with 5–10 carbon atoms in the molecule, in beer and fermented drinks and foods. Examples of acids with five carbon atoms in the molecule are (*Z*)-2-methylbut-2-enoic (angelic) (**8-62**) and (*E*)-2-methylbut-2-enoic acid (tiglic) acids, the precursor of which is threonine (**8-62**). Valine is converted into (*Z*)-3-methylbut-2-enoic (senecioic) acid with five carbon atoms (**8-62**). These acids, along with a number of other related acids, are a frequent component of pyrrolizidine alkaloids known as senecio alkaloids (see Section 10.3.2.1.3).

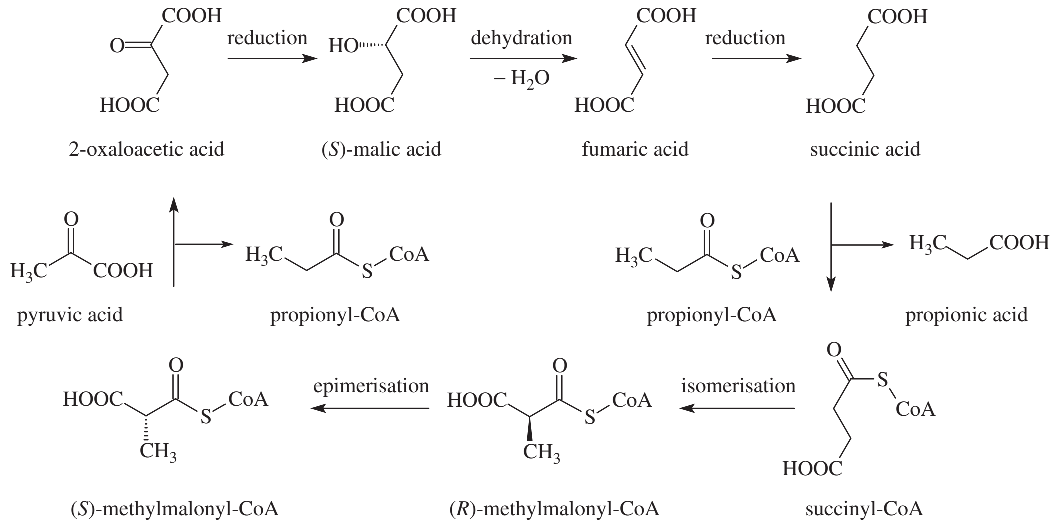
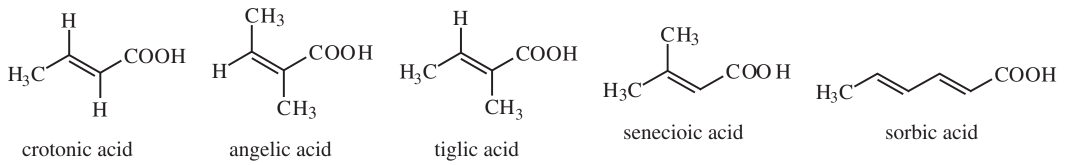


Figure 8.39 Mechanism of propionic acid fermentation.



8-62, unsaturated carboxylic acids

An important unsaturated acid is (2*E*,4*E*)-hexa-2,4-dienoic acid known as sorbic acid (**8-62**). It was first isolated from fruits of the rowan tree (*Sorbus aucuparia*, Rosaceae), where it results from dehydration and ring opening of (*S*)-2-methyl-2,3-dihydropyran-6-one, which is called parasorbic acid. Sorbic acid has antimicrobial effects and is therefore used as a preservative (see Section 11.2.1.1.2).

8.2.6.1.2 Aliphatic dicarboxylic and tricarboxylic acids

Aliphatic dicarboxylic and tricarboxylic acids are non-volatile compounds and may therefore just have a role as taste-active (acidic) food components.

The basic member of the homologous series of aliphatic dicarboxylic acids (**8-63**) is oxalic acid. The main precursor of oxalic acid is L-ascorbic acid; therefore oxalic acid is a normal constituent of fruits and vegetables (Figure 8.40). It occurs as a soluble potassium salt (e.g. in bananas) or a calcium salt (especially in fruits and vegetables) that is soluble in acidic and insoluble in neutral and alkaline media. Oxalic acid is categorised among the antinutritional compounds, as it interferes with the metabolism of calcium. Regulation

8-63, dicarboxylic acids

oxalic (ethanedioic), $n = 0$
malonic (propanedioic, 1,1-methanedicarboxylic), $n = 1$
succinic (butanedioic, 1,2-ethane dicarboxylic), $n = 2$
glutaric (pentanedioic, 1,3-propane dicarboxylic), $n = 3$
adipic (hexandioic, 1,4-butane dicarboxylic), $n = 4$
pimelic (heptanediic, 1,5-pentane dicarboxylic), $n = 5$
suberic(octanedioic, 1,6-hexane dicarboxylic), $n = 6$
azelaic(nonanedioic, 1,7-heptane dicarboxylic), $n = 7$
sebacic (decanedioic, 1,8-octane dicarboxylic), $n = 8$

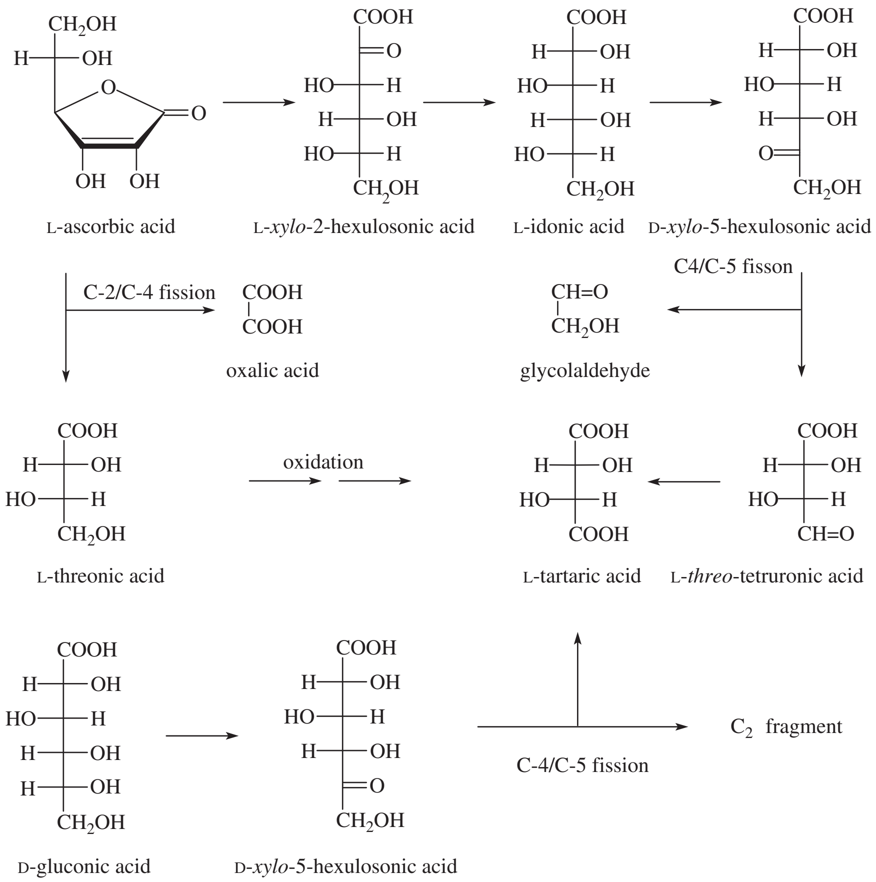


Figure 8.40 Biosynthesis of oxalic and L-tartaric acids.

of the calcium content in plants is based on the same principle. At higher concentrations, oxalic acid occurs in rhubarb and spinach, where its content can be somewhat decreased by blanching. A similar amount of oxalic acid is found in ripe carambola fruits (*Averrhoa carambola*, Oxalidaceae), tea and cocoa (Table 8.15). Tea is considered the major source of oxalic acid in countries where it is consumed in large quantities (such as the United Kingdom).

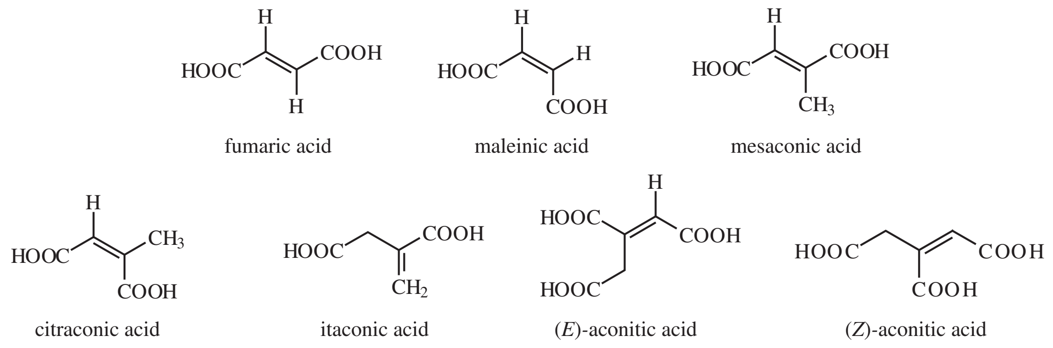
Higher saturated aliphatic dicarboxylic acids, such as malonic, succinic and glutaric acids and other higher homologues of oxalic acid, are found in many food raw materials and foods, but usually in smaller quantities. They are mainly intermediates of fatty acids biosynthesis, the citric acid cycle and other metabolic processes, but only succinic acid occurs in a somewhat larger amount in some fruits (e.g. in currants and strawberries). All the above mentioned dicarboxylic acids, including oxalic, adipic, pimelic, suberic acids and their higher homologues, are present in small amounts in wine and beer.

Among the unsaturated dicarboxylic acids, (*E*)-but-2-enedioic acid, known as fumaric acid (**8-64**) has a certain importance. It is formed in the ornithine cycle, and occurs in small quantities in virtually all products of animal and vegetable origin, and in greater quantities in some fungi. Fumaric acid arises as a non-enzymatic deamination product of aspartic acid. Along with isomeric (*Z*)-but-2-enedioic acid, known as maleinic (maleic) acid (**8-64**), fumaric acid forms by pyrolysis of malic acid during thermal processes, such as coffee roasting.

A higher homologue of fumaric acid is (*E*)-2-methylbut-2-enedioic acid, (*E*)-prop-1-ene-1,2-dicarboxylic acid, which is known trivially as mesaconic or methylfumaric acid (**8-64**). It occurs in significant quantities in sugar beets. It is used as a

Table 8.15 Oxalic acid contents in some foods.

Food	Content (mg/kg)	Food	Content (mg/kg)
Vegetables		Kiwi	180–450
Bamboo shoots	1 600–4 600	Oranges	62
Bell peppers	400	Orange juice	44
Broccoli	<100–500	Raspberries	22
Brussel sprouts	3 600	Strawberries	19
Cabbage	130–1250	***Cereals***	
Carrots	100–5000	Wheat (*Triticum aestivum*)	533
Celery (stems)	175	Rye	712
Cucumbers	20	Oats	163
Garlic	360	Basmati rice	172
Green beans	200–450	Maize	386
Onion	30–50	***Other foods***	
Potatoes	23	Wheat wholemeal flour	700
Parsley (curled)	1660	Rye wholemeal flour	289
Pea	500	Bread (white)	49
Radishes	3	Cornflakes	56
Rhubarb	2300–9600	Oatmeal porridge	10
Salad beet	300–1380	Lentils	1 180
Spinach	5400–9800	Tea (dry leaves)	3750–14 500
Tomatoes	50–100	Coffee (Nescafé powder)	570
Fruits		Cacao powder	6 230
Apples	15	Peanut butter	7 050
Banana	7	Beef (roasted)	4
Black currants	43	Milk	5
Carambola (star fruit)	500–9 600	Mushrooms (*Boletus edulis*)	136–536



8-64, unsaturated dicarboxylic and tricarboxylic acids

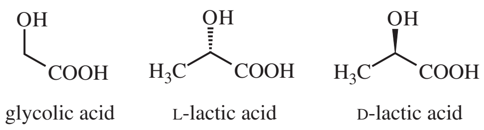
fire retardant. The *cis*-isomer of mesaconic acid is (*Z*)-2-methylbut-2-enedioic acid, also called (*Z*)-prop-1-ene-1,2-dicarboxylic acid, methylmaleic acid or citraconic acid (**8-64**). Blueberries contain higher amounts. 2-Methylidenebutanedioic acid, also known as prop-1-ene-2,3-dicarboxylic acid or itaconic acid (**8-64**), is produced, together with mesaconic and citraconic acids, by thermal degradation of citric acid. Citric acid degradation occurs, for example, during the roasting of coffee and the refining (deodorisation) of vegetable oils, where citric acid is used as an additive agent (about 100 mg of 20% citric acid per kg oil) to chelate traces of metals, resulting in increased oil stability during storage. The common, older name of these three acids is thus pyrocitric acid.

Tricarboxylic acid, known as aconitic acid (prop-1-ene-1,2,3-tricarboxylic acid, also known as achilleic or citridinic acid), occurs in two geometric isomers, the (*Z*)- and the (*E*)-isomers (**8-64**). (*Z*)-Aconitate is widespread as an intermediate produced in the isomerisation of citrate to D-isocitrate (catalysed by aconitase) in the citric acid cycle. About 5% aconitic acid is found in molasses from cane sugar production, where the (*E*)-isomer prevails, as it is formed by isomerisation of (*Z*)-aconitic acid at elevated temperatures and low pH. The amount of (*Z*)-aconitic acid in the growing cane is low, because it is used in the citric acid cycle and not stored in the plant. Decarboxylation of aconitic acid at elevated temperatures yields itaconic acid.

8.2.6.1.3 Aliphatic hydroxycarboxylic acids

Hydroxycarboxylic acids are mostly non-volatile and polar compounds that are not important as odour-active food components. Certain hydroxy acids are, however, the major substances influencing the sour taste of fruits, vegetables and other foods. Some of their reaction products are odour-active substances, especially lactones.

The simplest hydroxycarboxylic acid is glycolic (hydroxyethanoic) acid (**8-65**), which occurs in small amounts in most plant materials as a natural component. It also arises by the Cannizzaro reaction of glyoxal.



8-65, hydroxycarboxylic acids

The most important representative of this group of carboxylic acids is lactic (2-hydroxypropanoic) acid. (+)-L-Lactic acid is present in meat, that is (*S*)-2-hydroxypropanoic acid (**8-65**), which is formed in anaerobic glycolysis from glycogen. For example, fresh beef usually contains lactic acid at a level of 0.2–0.8%.

Homofermentative lactic acid bacteria (such as *Lactococcus lactis* and *Streptococcus lactis*) produce (+)-L-lactic acid (e.g. in sour cream). Both isomers, (+)-L-lactic acid and (−)-D-lactic acid (**8-65**), are formed during milk fermentation by heterofermentative bacteria (lactic acid bacteria are mostly heterofermentative bacteria) and lactic acid thus also occurs as a racemate in sauerkraut, pickled cucumbers, olives and silage. For example, bacteria of the genus *Leuconostoc* produce D-lactic acid, while bacteria *Pediococcus acidilactici* and other bacteria produce racemic lactic acid. The content of lactic acid in dairy products is 0.5–1.0%. L-Lactic acid in yoghurt represents about 54% and in sour cream 96% of the total lactic acid content. The total lactic acid content in sauerkraut is 1.5–2.5%, in fermented cucumbers it ranges from 0.5 to 1.5% and fermented green olives contain 0.8 to 1.2% lactic acid.

Fermentation with homofermentative lactic acid bacteria involves phosphorylation of α-lactose to α-lactose 6′-phosphate and hydrolysis to α-D-galactose 6-phosphate and β-D-glucose. α-D-Galactose 6-phosphate is then degraded via D-tagatofuranose 6-phosphate and D-tagatofuranose 1,6-bisphosphate to D-glyceraldehyde 3-phosphate. β-D-Glucose is phosphorylated to β-D-glucose 6-phosphate. All sugars are then activated by different mechanisms and converted into D-fructose 1,6-bisphosphate, which is degraded, via D-glyceraldehyde 3-phosphate and other metabolites, to pyruvic acid, which is reduced to L-lactic acid, virtually the only product of homofermentative lactic acid fermentation[3] (Figure 8.41).

Heterofermentative lactic acid bacteria produce L- and D-lactic acids, ethanol, carbon dioxide and a small amount of acetic acid from glucose and fructose. Sugars are phosphorylated in the bacterial cells: glucose to β-D-glucose 6-phosphate by glucokinase and fructose usually to D-fructose 1-phosphate by fructokinase.

[3] The reaction sequences involve phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, L-lactate dehydrogenase, galactokinase, UDP-galactose pyrophosphorylase, UDP-galactose 4-epimerase, UDP-glucose pyrophosphorylase, phosphoglucomutase, glucokinase, fructokinase, phosphoglucose isomerase, fructose bisphosphate aldolase, 1-phosphofructokinase, phospho-β-galactosidase, D-galactose-6-phosphate isomerase, D-tagatose-6-phosphate kinase and tagatose-1,6-bisphosphate aldolase.

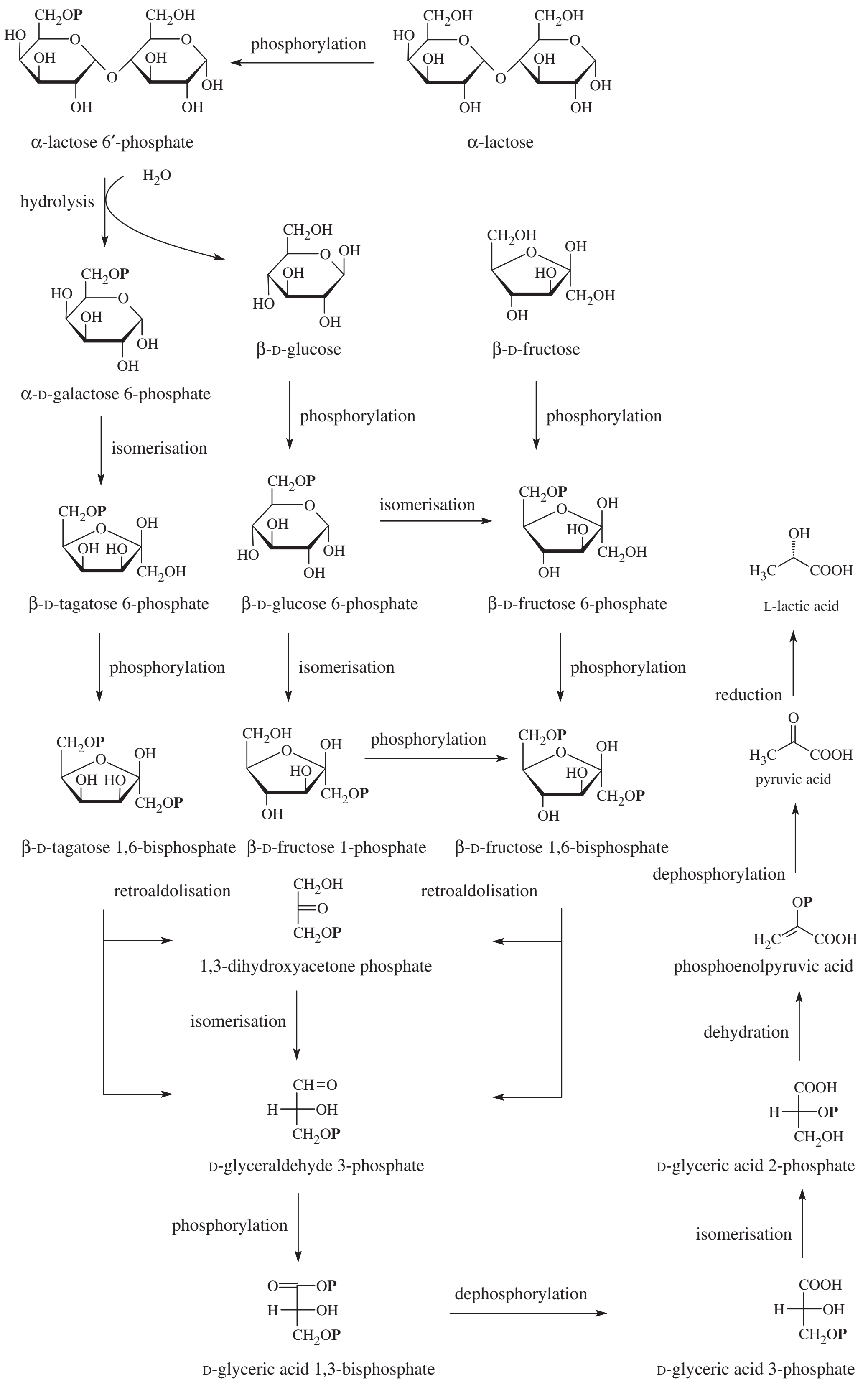
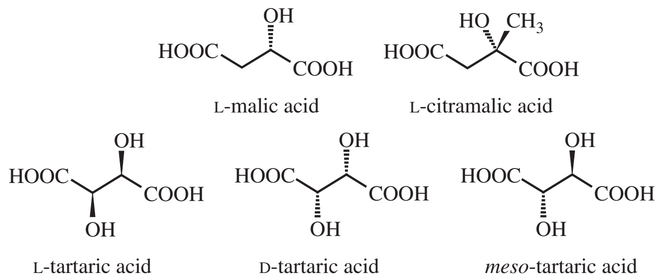


Figure 8.41 Mechanism of homofermentative lactic acid bacteria fermentation (**P** = phosphate residue).

D-Fructose 1-phosphate, with the catalysis of glucose-6-phosphate isomerase, isomerises to β-D-glucose 6-phosphate (which is sometimes produced directly), which is further metabolised (Figure 8.42; structures of compounds are shown in Figure 8.41). Saccharose is first hydrolysed to glucose and fructose by invertase, and maltose is hydrolysed to glucose by maltase.

Polycarboxylic hydroxy acids are important carriers of the acidic taste of foods. Dicarboxylic hydroxy acids primarily include malic (hydroxysuccinic) acid (**8-66**) occurring only as (−)-L-isomer that is the (*S*)-isomer, which results as an intermediate in the citric acid



8-66, hydroxydicarboxylic acids

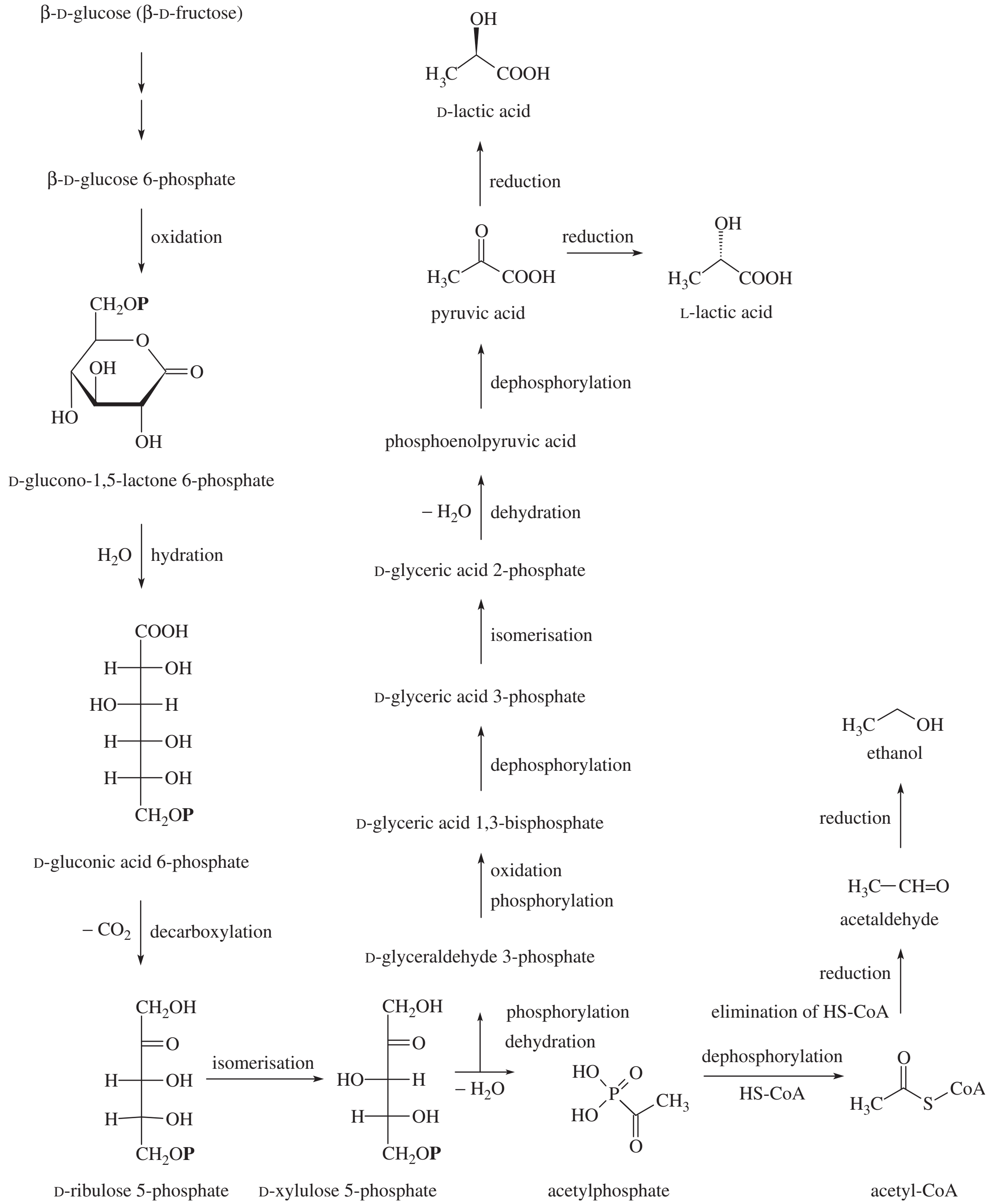


Figure 8.42 Mechanism of heterofermentative lactic acid bacteria fermentation (**P** = phosphate residue).

cycle. It is found in abundance primarily in fruits and vegetables. Malic acid is used as a food additive (acidulant), and is synthesised from fumaric acid using the bacteria *Lactobacillus brevis* or yeasts of the genus *Candida*.

In the skins of apples, pyruvic acid is the precursor of (+)-L-citramalic acid, which is also known as (*S*)-2-hydroxy-2-methylsuccinic acid or (*S*)-2-methylmalic acid (**8-66**). During fruit ripening, citramalic acid is oxidised to 2-oxobutyric acid and its decarboxylation yields acetone, which is a component of apple odour. The same isomer of citramalic acid is produced by some enterobacteria as a degradation product of glutamic acid to pyruvic acid. The (*R*)-isomer of citramalic acid is an intermediate in the biosynthesis of isoleucine in spirochetes.

Tartaric (2,3-dihydroxysuccinic) acid is an important representative of dihydroxydicarboxylic (aldaric) acids and the most prominent acid in wine. In nature it occurs almost exclusively as (+)-L-tartaric acid (**8-66**), also known as dextrotartaric acid or L-threaric acid, which is (2*R*,3*R*)-isomer. Occasionally tartaric acid occurs as (−)-tartaric, laevotartaric acid, which is the (2*S*,3*S*)-isomer, also known as D-threaric acid (**8-66**). An optically inactive racemic mixture of both isomers, called racemic (uvic) acid, has been demonstrated in grape juice, the optically active symmetric (2*R*,3*S*)-tartaric acid (**8-66**) called *meso*-tartaric or erythraric acid does not occur in nature. Synthetic racemic and *meso*-tartaric acids are used as acidulants.

L-Tartaric acid is found in wines as the poorly soluble potassium hydrogen tartrate salt (potassium bitartrate) that often crystallises in young wines and when the wine is cooled as cream of tartar, known in winemakers' jargon as tartrates. The crystals are harmless, but their presence is generally undesirable to consumers. The cream of tartar formation can be prevented by cold stabilisation of wines.

L-Tartaric acid biosynthesis starts from L-ascorbic acid or from D-gluconic acid (Figure 8.40). In grapes and other plants of the Vitaceae family, tartaric acid arises from the ascorbic acid intermediate D-*xylo*-5-hexulosonic acid via the cleavage between carbons C-4 and C-5. The second option, which is apparently used by most plants (the reaction is catalysed by ascorbate 2,3-dioxygenase), is the ascorbic acid cleavage between carbons C-2 and C-3, which simultaneously yields oxalic acid. The third possibility for the biosynthesis of L-tartaric acid from D-gluconic acid is used mainly by plants of the Fabaceae family.

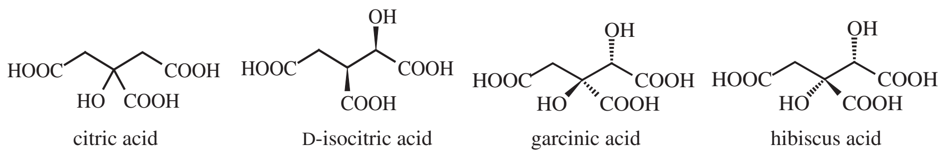
The optically inactive citric acid (**8-67**) is the most important representative of tricarboxylic hydroxy acids. Citric acid, as well as malic acid, occurs in many fruits (especially in lemons). Industrially, it is obtained from lemon juice or produced by fermentation of molasses using fungi *Aspergillus niger*. Along with malic acid, citric acid is used as an additive in widely different canning products and soft drinks as an acidulant, but also serves in refination (degumming) of vegetable oils and for other purposes.

The malic and citric acids contents in fruits and vegetables are summarised in Table 8.16. In addition to these major acids, other carboxylic acids are also present in smaller amounts, such as tartaric acid, which occurs in higher amounts in grapes. Generally, fruits and vegetables contain, as well as malic, citric and tartaric acids, a number of other acids of which succinic, fumaric, quinic, pyruvic and oxalic acids (together with ascorbic acid) predominate. For example, depending on variety, growing stage and many other factors, the total amount of organic acids in carrots is about 2000–3000 mg/kg. After harvest, isocitric and malic acids represent about 90–95% of the total acids. The content of isocitric and malic acids is approximately 1000 mg/kg and 800 mg/kg, respectively. Tartaric (18–55 mg/kg), succinic (22–130 mg/kg), fumaric (5–8 mg/kg), quinic (42–60 mg/kg) and oxalic acids (100 mg/kg) are found in smaller amounts, as are some other acids in lower amounts.

An isomer of citric acid is (1*R*,2*S*)-1-hydroxypropan-1,2,3-tricarboxylic acid (**8-67**), also known as D-isocitric acid (D-*threo* isomer), which is another intermediate of the citric acid cycle. This acid is the dominant acid in blackberries (Table 8.17) and occurs in other fruits in small or insignificant amounts (e.g. in apples). The isocitric acid content is one of the most important markers in the estimation of the proportion of fruit in coloured fruit mixtures, for example in mixtures of blackcurrants, strawberries and raspberries with apples. The concentration ratio of citric acid to isocitric acid is also employed for the detection of adulteration, and can reveal the addition of synthetic citric acid to optimise the taste of fruit drinks, especially in the case of added sugar.

The hydroxy derivative of citric acid, (1*S*,2*S*)-1,2-dihydroxypropane-1,2,3-tricarboxylic acid, also known as garcinic or (−)-hydroxycitric acid (**8-67**), together with the corresponding γ-lactone, occurs in amounts of 16–18% of dry matter in the skin of the Southeast Asian fruit of *Garcinia gummi-gutta* (syn. *G. cambogia*, Clusiaceae) tree, known as the Malabar tamarind. This fruit is related to the more common mangosteen (*G. mangostana*). Extracts from the fruit rind, called citrin, are used as dietary supplements for weight loss because garcinic acid inhibits the activity of ATP citrate lyase, an enzyme involved in conversion of glucose into fat, thereby reducing the extent of lipogenesis by increasing the formation of glycogen in the liver and suppression of appetite.

The garcinic acid isomer, (1*S*,2*R*)-1,2-dihydroxypropane-1,2,3-tricarboxylic acid, also known as hibiscus acid (**8-67**), is located in the spicy leaves of hibiscus called roselle (*Hibiscus sabdariffa*, Malvaceae), which is prepared like spinach in the tropics. Its fleshy flower calyces, containing intense staining anthocyanine pigments,



8-67, hydroxytricarboxylic acids

Table 8.16 Malic and citric acids contenrs in fruits and vegetables.

	Content (mg/kg)			Content (mg/kg)	
Fruits	**Malic acid**	**Citric acid**	**Vegetables**	**Malic acid**	**Citric acid**
Apples	2000–13 000	75–100	Asparagus	600–670	430–2000
Apricots	8000–14 100	7 000	Broccoli	1200	2 100
Bananas	2770	680	Cabbage	500–2000	700–4510
Black currant	4000–4400	25 000–31 600	Carrots	800–5200	40–930
Gooseberry	7000–000	6000–8000	Cauliflower	3900	2100
Grapefruits	400–600	11 900–21 000	Cucumbers	2400	100–3260
Grapes[a]	1680–15 360	305–1160	Garlic	2400	190
Lemons	1700–3 000	40 000–43 800	Green beans	1120–1300	300–340
Oranges	600–2 000	5600–9800	Green peas	750–1730	1100–1790
Peaches	3000–7700	<2000	Lettuce	920–2430	120–270
Pears	1000–4150	1800	Onion	1550–1960	230–1100
Pineapple	1000–5000	4000–12 000	Rhubarb[b]	9100–10 250	1350–1370
Red currant	1000–3000	12 000–20 200	Spinach	360–640	82–101
Strawberries	900–2000	6000–11 000	Tomatoes	1350–4700	8800–26 300

[a] Tartaric acid in the amount of 4190–13 510 mg/kg.
[b] Oxalic acid in the amount of 2300–9600 mg/kg.

Table 8.17 Isocitric acid content in selected fruits and the ratio of citric acid and isocitric acid in authentic fruit juices.

Fruits	Isocitric acid (mg/kg)	Citric acid/isocitric acid ratio
Apples	<5	0.0025–0.0004
Apricots	75–200	15–130
Black currant	160–500	80–200
Blackberries	8 000–10 000	0.5–3.0
Grapefruits	140–350	50–95
Oranges	65–200	<130
Peaches	30–160	15–100
Pears	<40	0.004–0.009
Raspberries	60–220	80–200
Strawberries	30–90	100–230

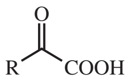
are increasingly used as food colourings and are often added to fruit and herbal teas.

An unusual citric acid derivative is toxic agaricinic (agaric) acid, which occurs in some fungi (see **10-241**). Foods also contain numerous hydroxy acids derived from sugars.

8.2.6.1.4 Aliphatic oxocarboxylic acids

2-Oxocarboxylic acids are major products of the metabolism of proteins, lipids and carbohydrates, and also arise as intermediates in fermentation processes, as products of other enzymatic reactions, which are not linked with the metabolism of major nutrients, and also as products of non-enzymatic reactions. They usually occur in low concentrations in all foods of animal and vegetable origin. Oxoacids are polar and non-volatile compounds, but some of their reaction products are important odour-active substances in foods. An example is 5-ethyl-3-hydroxy-4-methyl-5*H*-furan-2-one (abhexon), which appears in acid protein hydrolysates from threonine via 2-oxobutyric acid.

The simplest 2-oxocarboxylic acid is glyoxylic acid (**8-68**), which occurs in the immature fruits of many plants. Glyoxylic acid is produced as the main intermediate of the glyoxylic acid cycle (ongoing in plants and microorganisms) and is also formed by other biochemical reactions, such as degradation of purine bases,

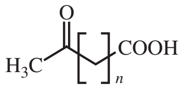


8-68, 2-oxoacids

glyoxylic, R = H
2-oxopropionic (pyruvic), R = CH_3
2-oxobutyric, R = CH_2CH_3
2-oxosuccinic (oxaloacetic), R = CH_2COOH

4-hydroxyproline and other compounds. The key compound of metabolism of carbohydrates, lipids and proteins in animals and plants is pyruvic (2-oxopropionic) acid (**8-68**) that also occurs in fruits and vegetables. It is similarly formed by enzymatic cleavage of alliin and other *S*-alk(en)yl-L-cysteine derivatives in garlic, onion and other plants. Some higher homologues of glyoxylic acid, such as 2-oxobutyric (**8-68**), 2-oxovaleric, 2-oxoisovaleric, 2-oxoisokapronic and 2-oxo-3-methylvaleric acids, are precursors of fusel oil alcohols in alcoholic beverages.

Foods also contain various oxodicarboxylic and oxotricarboxylic acids that arise predominantly in the citric acid or glyoxylic acid cycles. A common acid is oxaloacetic (2-oxosuccinic) acid (**8-68**). 3-Oxoacids are usually represented by acetoacetic (3-oxobutanoic) acid (**8-69**). The main non-volatile dicarboxylic acids, hydroxy acids and oxoacids contents in beer are given in Table 8.18 as an example.

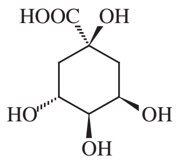


8-69, acetoacetic acid, *n*= 1
laevulinic acid, *n*= 2

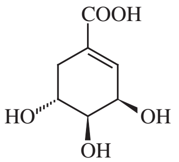
It is common to find laevulinic (4-oxopentanoic) acid (**8-69**) in foods containing carbohydrates, as it is one of the final dehydration products of hexoses via 5-hydroxymethylfuran-2-carbaldehyde in acidic media. It is present in very high concentrations (about 1–2% w/w) in acid hydrolysates of proteins.

8.2.6.1.5 Alicyclic carboxylic acids

One of the most important substances derived from tetrahydroxycyclohexane is (1*S*,3*R*,4*S*,5*R*)-1,3,4,5-tetrahydroxycyclohexane-1-carboxylic acid, known as (−)-L-quinic acid (**8-70**). The trihydroxycyclohexene derivative is related (3*R*,4*S*,5*R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid known as (−)-L-shikimic acid (**8-71**), which is a key intermediate in the biosynthesis of phenolic compounds. As free acids, but mainly as esters (depsides) with (*E*)-cinnamic acids (caffeic, ferulic and 4-coumaric acids), both compounds are the major components of coffee beans. In smaller amounts they are found in tea leaves, cocoa beans, fruits, vegetables, potatoes and other plant materials. In the juices of berries (e.g. gooseberries, currants and blueberries) and mango, these acids occur in hundreds of thousands of mg/l, but in other fruits their content is about ten times lower. For example, the quinic acid content can be used to differentiate the North American blueberry products (juices or jams) made from northern high bush blueberries (*Vaccinium corymbosum*, Ericaceae), which contain from 0.37 to 2.53 g/kg quinic acid, from European blueberry (bilberry) products prepared from *V. myrtillus* berries that contain 3.74–7.84 g/kg quinic acid.



8-70, L-quinic acid



8-71, L-shikimic acid

Table 8.18 Major dicarboxylic acids, hydroxy acids and oxoacids contents in beer.

Acid	Content (mg/kg)	Acid	Content (mg/kg)
Succinic	5–166	L-Malic	0–213
L-Lactic	2–362	Citric	5–262
D-Lactic	0–372	Pyruvic	5–230

8.2.6.1.6 Aromatic carboxylic acids

The simplest aromatic carboxylic acid is benzoic acid (**8-72**), which is relatively widespread in plant materials, mainly as 1-*O*-benzoyl-β-D-glucopyranose (see Section 4.3.3.3), but both compounds are non-volatile polar substances that do not have any impact on food flavour. In essential oils, benzoic acid is found in the form of flavour-active esters. Benzoic acid arises from cinnamic acid or cinnamoyl-CoA through side-chain shortening by a C_2 unit. Its content in fruits and vegetables is generally very low, at around 0.05%. In cranberries it is present predominantly as 6-*O*-benzoyl-β-D-glucopyranose, known as vaccinin (see **4-92**), in an amount of around 0.2%. Free benzoic acid is also present in a very small amount (about 0.0015%) in yoghurts where it results from hippuric acid hydrolysis. It is often also added to foods as a preservative. A typical example of a preserved product is table mustard, where concentrations of benzoic acid typically reach 1000 mg/kg.

Benzoic acids substituted by hydroxyl and methoxyl groups (**8-72**) are produced through different biochemical mechanisms. In the shikimate pathway via shikimic acid (its precursors or metabolites), 2-hydroxybenzoic (salicylic), 4-hydroxybenzoic, 2,3-dihydroxybenzoic, 3,4-dihydroxybenzoic (protocatechuic) and 3,4,5-trihydroxycarboxylic (gallic) acids are formed. Salicylic acid occurs in small quantities (0.3–3 mg/kg) in different types of fruit. It is also present in the form of glycosides and esters. Salicylic acid participates in thermogenesis, the metabolism of minerals, induces plant flowering, affects ethylene biosynthesis, resistance to pathogens and other processes. A mixture of 6-alkatrienyl, 6-alkadienyl, 6-alkenyl and 5-alkyl substituted salicylic acids, collectively known as anacardic acid, occur in the cashew nutshell liquid at a level of 60–65%, together with decarboxylated products known as cardol (see Section 10.2.3.4) and the corresponding phenols that are called cardanol (see Section 8.2.8.1). The main components are (8*Z*,11*Z*)-2-hydroxy-6-(pentadeca-8,11,14-trien-1-yl)benzoic acid, (8*Z*,11*Z*)-2-hydroxy-6-(pentadeca-8,11-dien-1-yl)benzoic acid, (8*Z*)-2-hydroxy-6-(pentadeca-8-en-1-yl)benzoic acid and 2-hydroxy-6-pentadecylbenzoic acid. Anacardic acid possesses antimicrobial, antiacne and other medicinal properties. Owing to its antimicrobial effects, 4-hydroxybenzoic acid has found use as a preservative mainly in the form of esters (so-called parabens). 2,3-Dihydroxybenzoic acid is a part of various bacterial

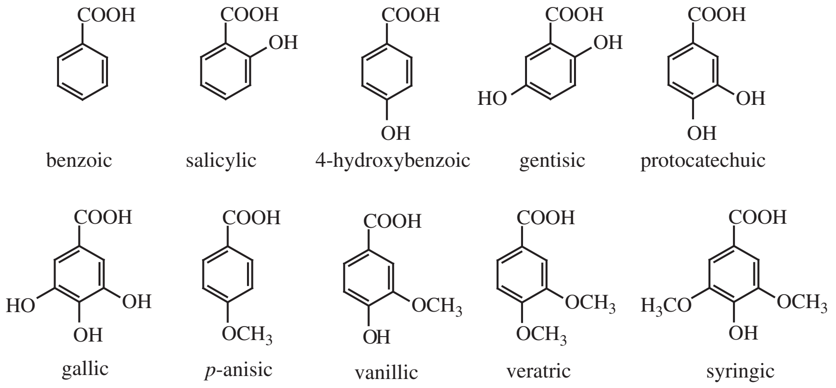
siderophores (see Section 6.3.6.1.2), 2,4-dihydroxybenzoic (β-resorcylic or *p*-hydroxysalicylic) acid is a common phenolic acid in wheat and rye, 2,5-dihydroxybenzoic (gentisic) acid occurs in wine and 3,4-dihydroxybenzoic (protocatechuic) acid is an effective antioxidant in fruits, vegetables and also *in vivo*. Gallic acid is present in foods of plant origin in small amounts as the free acid. The frequently occurring gallic acid dimer is known as ellagic acid. Gallic acid is usually present in a number of other related compounds, which, like gallic acid, are components of hydrolysable tannins (see Section 8.3.5.1).

Analogously, various hydroxy- and methoxy substituted benzoic acids are formed in plants by shortening of the side chain of hydroxy- and methoxy substituted cinnamic acids by C_2 units (**8-72**). The hydroxy- and methoxy substituted precursors are biosynthesised from cinnamic acid and also arise as lignin degradation products with some bacteria and fungi. Relatively common substances are 4-hydroxybenzoic and 2,5-dihydroxybenzoic (gentisic) acids. The latter acid occurs in significant quantities, for example in cocoa, and is used as an antioxidant in some pharmaceutical products. 4-Methoxybenzoic (*p*-anisic) acid is found in anise, 4-hydroxy-3-methoxybenzoic (vanillic) acid accompanies vanillin in vanilla pods, other acids, such as 3-hydroxy-4-methoxybenzoic (isovanillic), 3,4-dimethoxybenzoová (veratric) acid, 3,4-dihydroxy-5-methoxybenzoic (3-*O*-methoxygallic) acid and 4-hydroxy-3,5-dimethoxybenzoic (syringic) acid occur in small amounts in all foods of plant origin.

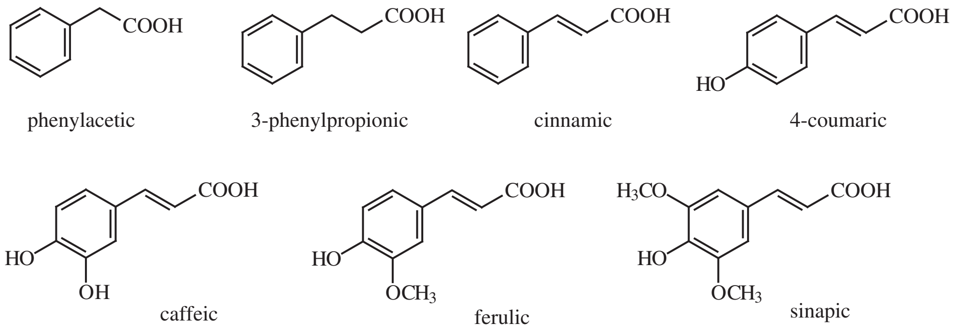
Higher homologues of benzoic acid are phenylacetic and 3-phenylpropionic acids (**8-73**), which result from the microbial degradation of organic matter (such as lignin) and by oxidation of the corresponding aldehydes. Phenylacetic acid has a low odour threshold concentration and is reminiscent of honey. In the form of esters it is often present in some essential oils, and is also used in perfumery. 3-Phenylpropionic acid is found at low concentrations in cheeses produced in the presence of molds.

The most common unsaturated aromatic carboxylic acid is cinnamic acid (**8-73**). In cinnamon and other spices the (*E*)-isomer predominates. Cinnamic acid oxidation products are 4-hydroxycinnamic acid (also known as 4-coumaric or *p*-coumaric acid) and 3,4-dihydroxycinnamic acids, which is called caffeic acid. A 3-methoxy derivative of caffeic acid is ferulic acid and the 3,5-dimethoxy derivative is known as sinapic acid.

The content of the major phenolic acids in different fruits differs qualitatively and quantitatively, and changes significantly during fruit growth and ripening. For example, apples at harvest time contain in their fresh weight about 12 mg/kg *p*-coumaric, 85 mg/kg caffeic and 4 mg/kg ferulic acid. At maturity, the content of these acids is 1.9, 10.4 and 0.4 mg/kg, respectively. Ripe strawberries contain as the main acid *p*-coumaric acid (175 mg/kg) and present in larger quantities are gallic (121 mg/kg), 4-hydroxybenzoic acid (108 mg/kg), caffeic (39 mg/kg) and vanillic (34 mg/kg) acids. In cereal grains, phenolic acids are concentrated in the aleurone cells and other outer layers of the grain that form the bran on



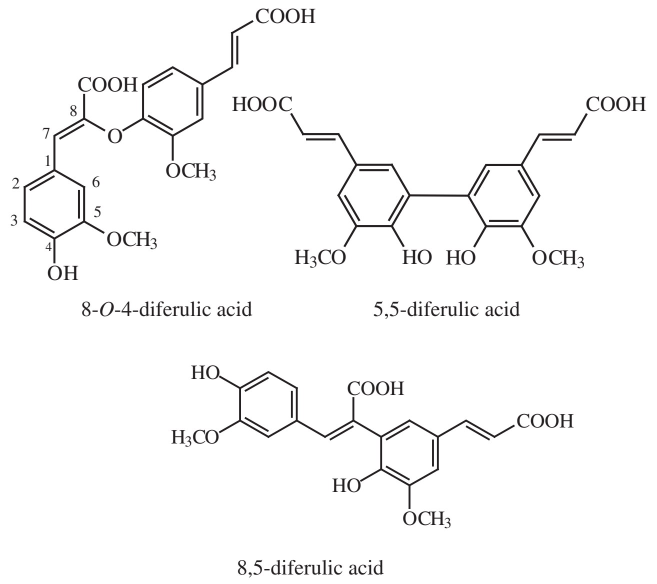
8-72, benzoic acid and substituted benzoic acids



8-73, higher homologues of benzoic acid and common (*E*)-cinnamic acids

milling. They are present mainly in the bound forms, linked to cell wall structural components, such as cellulose, lignin and proteins. The majority of the feruloyl groups are attached to the cell wall arabinoxylan via the carboxyl groups acylating the primary hydroxyl groups at the C-5 positions of the α-L-arabinofuranosyl residues. It has been shown that ferulates can form dimers through oxidative cross-linking between esterified feruloyl groups, which may serve to cross-link cell-wall polysacharides. The main ferulic acid dehydrodimer is 8-*O*-4-diferulic acid. Examples of other dehydrodimers are 5,5- and 8,5-diferulic acids (**8-74**). Sinapic acid dehydrodimers and sinapate-ferulate heterodimers have also been identified in cereal grain dietary fibers. The phenolic acids contents in different wheat species and rye cultivars are given as an example in Table 8.19. Their content in beer is illustrated in Table 8.20.

Cinnamic acids are present in plant tissues as free substances, but in larger quantities are found as esters, amides or glycosides. An example is a glycoside with antioxidant effects, (*E*)-1-*O*-sinapoyl-β-D-glucopyranose (**8-75**), occurring in germinating seeds of cruciferous plants (Brassicaceae), where it is produced from sinapine. An effective antioxidant in olives (*Olea europea*, Oleaceae) and also of some mullein species (*Verbascum sinuatum*, Scrophulariaceae) and perhaps of other plants, is verbascoside (**8-76**), a glycoside derived from 3,4-dihydroxyphenylethanol (hydroxytyrosol). The sugar component is the disaccharide 6-deoxy-β-D-glucopyranosyl-(1→3)-β-D-glucopyranose (chinovose) esterified by (*E*)-caffeic acid.



8-74, ferulic acid dimers

8.2.6.1.7 Heterocyclic carboxylic acids

In addition to benzoic and cinnamic acids, plant materials and foods contain a number of heterocyclic carboxylic acids, the most

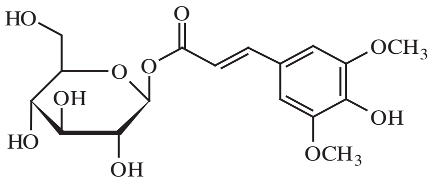
Table 8.19 Free, conjugated and bound phenolic acid in wheat and rye.

	Wheat (mg/kg dry matter)			Rye (mg/kg dry matter)		
Phenolic acid	Free	Conjugated	Bound	Free	Conjugated	Bound
Benzoic acids						
4-Hydroxybenzoic	0-0.10	2.3-16.2	0-8.3	-	5.5-13.0	1.2-14.0
2,4-Dihydroxybenzoic	0-4.6	5.2-147	0-215	0.5-5.0	44.6-107	0-109
Vanillic	0-4.4	7.0-24.5	1.7-9.0	1.1-1.7	4.6-7.8	1.4-10.4
Syringic	0-5.1	1.2-22.0	0.2-13.4	1.1-2.3	0.2-3.0	0.3-12.4
Cinnamic acids						
2-Hydroxycinnamic	0.1.1	1.3-3.6	2.4-7.4	0.1-0.5	1.6-2.4	3.3-25.7
4-Coumaric	0-2.3	1.7-14.6	2.4-19.1	0.7-1.7	7.4-25.8	2.7-59.5
Caffeic	0-4.3	-	-	3.1-3.7	-	-
Sinapic	0-12.3	21.5-137	12.9-40.0	8.8-9.6	51.6-91.4	26.1-60.1
Ferulic	1.2-6.2	9.4-87.8	162-721	3.0-6.4	34.8-123	146-521
Total acids	300->1000			491-1082		
Total free acids	3-30			11-29		
Total conjugated acids	76-416			153-349		
Total bound acids	208-964			216-711		

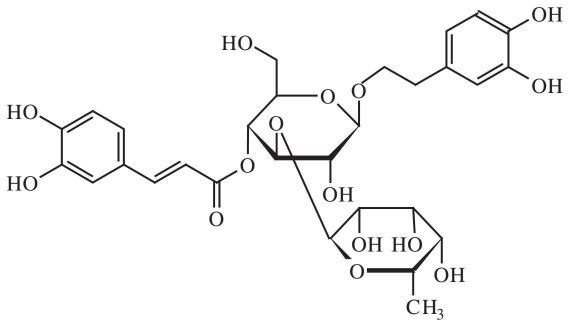
Table 8.20 Phenolic acids content in beer.

Acid	Content (mg/kg)	Acid	Content (mg/kg)
Benzoic	0.4	Phenylpropionic	<0.1
Salicylic	0.02–3.1	Phenylpyruvic	0.8–1.0
4-Hydroxybenzoic	0.1–15.9	Cinnamic[a]	0.6–26.1
Gentisic	0.3–4.6	*o*-Coumaric	0.2–0.3
Protocatechuic	1.0–19.0	*m*-Coumaric	<0.1
Vanillic	0.3–1.1	*p*-Coumaric	1.2–7.0
Syringic	1.3–7.0	Caffeic	2.0–8.0
Gallic	1.1–29.2	Ferulic	2.2–20.8
Phenylacetic	0.5–0.9	Sinapic	0.7–3.9

[a]The (*Z*)-isomer content is <0.01 mg/l.

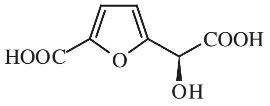


8-75, (*E*)-1-*O*-sinapoyl-β-D-glucopyranose

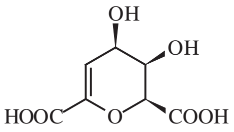


8-76, verbascoside

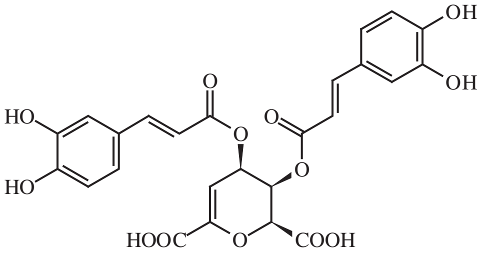
common of which are carboxylic acids with substituted furan and 4*H*-pyran (γ-pyran) skeletons. Examples of these acids are furan-2-carboxylic acid, which arises, for example, by oxidation of furan-2-carbaldehyde and by degradation of ascorbic (dehydroascorbic) acid. Analogously, oxidation of 5-hydroxymethylfuran-2-carbaldehyde yields 5-hydroxymethylfuran-2-carboxylic acid. A number of biologically active C_7 dicarboxylic acid are synthesised by plants across a range of plant families, such as carrots, wheat, sunflower, sugar beet and tobacco, as secondary metabolites . Examples of these carboxylic acids are vasorelaxation active (+)-osbeckic acid found, for example, in buckwheat, (−)-daucic acid from carrot with antioxidative activity, chelidonic acid that is active in allergic reactions, first identified in celandine (*Chelidonium majus*, Papaveraceae) sap and antiinflammatory and antibacterial meconic acid, which is found in plants of the Papaveraceae family, such as *Papaver somniferum* (opium poppy) (**8-77**). These acids occur either free or esterified with various phenolic acids. An example of such esters is 4,5-di-*O*-caffeoyldaucic acid found in tubers of sweet potato (*Ipomoea batatas*, Convolvulaceae).



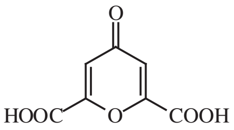
(+)-osbeckic acid



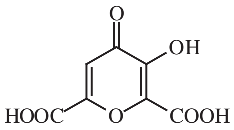
(−)-daucic acid



4,5-di-*O*-caffeoyldaucic acid



chelidonic acid



meconic acid

8-77, *O*-heterocyclic carboxylic acids

Several carboxylic acids are also derived from various *S*- and *N*-heterocyclic compounds. Many of them are natural biologically active compounds and other acids are formed during food processing. Examples are some vitamins, such as biotin, lipoic acid, nicotinic acid, 4-pyridoxic acid or orotic acid. Yeast autolysates, for example, contain a number of carboxylic acids derived from thiophene.

8.2.6.2 Properties and reactions

8.2.6.2.1 Properties

In solutions and also in the gaseous state, carboxylic acids are molecules associated by hydrogen bonds. The acidity of carboxylic acids is conditioned, according to electron theory, by the presence of carbonyl groups, which facilitate the dissociation of the hydroxyl to carboxylate anion:

$$R-COOH + H_2O \rightleftharpoons R-COO^- + H_3O^+$$

The strength of acids (their acidity) is expressed by their dissociation constants K_a, which depend on temperature and type

Table 8.21 Dissociation constants of acids.

Acid	pK_a value at 25 °C			Acid	pK_a value at 25 °C		
	pK_{a1}	pK_{a2}	pK_{a3}		pK_{a1}	pK_{a2}	pK_{a3}
Formic	3.75	-	-	Glyoxylic	2.98	-	-
Acetic	4.53	-	-	Quinic	3.58	-	-
Propionic	4.87	-	-	Shikimic	4.76	-	-
Butyric	4.83	-	-	Oxalic	1.20	3.67	-
Isobutyric	4.84	-	-	Succinic	4.22	5.70	-
Valeric	4.81	-	-	Fumaric	3.09	4.60	-
Sorbic	4.76	-	-	Malic	3.46	5.21	-
Benzoic	4.19	-	-	Tartaric	2.98	4.34	-
Glycolic	3.70	-	-	Citric	2.79	4.30	5.65
Lactic	3.83	-	-	Isocitric	3.28	4.71	6.39
Pyruvic	2.39	-	-	Phosphoric	2.15	7.10	12.40

of solvent (its relative permittivity).[4] The acidity of carboxylic acids lies between the strong mineral acids and carbonic acid. An overview of pK_a values of some acids is given in Table 8.21.

The dissociation of acids is influenced considerably by other functional groups in the vicinity of the carboxyl group. If in the neighbourhood there is a group repelling electrons (+I effect), such as alkyl, the acid is a weak acid (the K_a value is lower). Electronegative substituents in the vicinity of the carboxyl group, such as hydroxyl or carbonyl group, facilitate dissociation (–I effect). The inductive effects are less observable (or not at all) with increasing distance from the carboxyl group. The strongest aliphatic monocarboxylic acid is therefore formic acid, and hydroxycarboxylic acids and oxocarboxylic acids are stronger acids than the corresponding unsubstituted carboxylic acids.

8.2.6.2.2 Reactions

The most common chemical transformations of carboxylic acids in food are reactions caused by the cleavage of the O–H bond of the carboxylic group (salt formation), esterification, decarboxylation and reactions ongoing on the hydrocarbon residue.

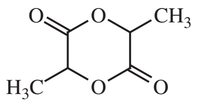
Reactions of aliphatic unsubstituted carboxylic acids

The most important reaction is the formation of salts and esters. Acids usually eliminate carbon dioxide with difficulty. The prominent reaction of higher fatty acids is oxidation of the hydrocarbon chain.

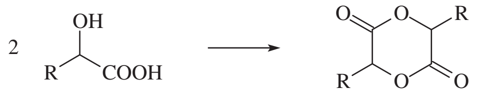
[4]Dissociation constants of acids $K_a = \frac{[RCOO^-][H_3O^+]}{[RCOOH]}$; values in parentheses are the molar concentrations. The K_a value includes the almost constant molar concentration of water. Negative decimal logarithms of dissociation constants K_a, which are referred to as pK_a values, are used for practical purposes.

Reactions of hydroxycarboxylic acids

Heating aliphatic α-hydroxycarboxylic acids leads to their dehydration and the formation of six-membered cyclic esters, derivatives of 1,4-dioxane, that are called lactides (Figure 8.43). Lactic acid is an α-hydroxy acid, which forms a cyclic diester 3,6-dimethyl-2,5-dioxo-1,4-dioxane (**8-78**) by heating. Heating of 3-hydroxy acids (β-hydroxy acids) leads to elimination of water to form α,β-unsaturated (alk-2-enoic) acids (Figure 8.44). Heating of γ- and δ-hydroxy acids leads to intramolecular esterification to form **lactones** (γ- and δ-lactones, Figure 8.45 and Figure 8.46). Higher lactones than δ-lactones do not result from hydroxy acids.

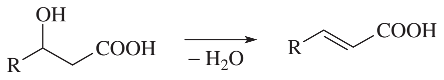


8-78, 3,6-dimethyl-2,5-dioxo-1,4-dioxane



2-hydroxycarboxylic acid — lactide (2,5-dioxo-1,4-dioxane)

Figure 8.43 Dehydration of α-hydroxycarboxylic acids.



3-hydroxycarboxylic acid — alk-2-enoic acid

Figure 8.44 Dehydration of β-hydroxycarboxylic acids.

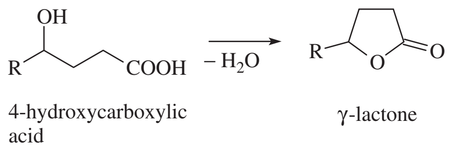


Figure 8.45 Dehydration of γ-hydroxycarboxylic acids.

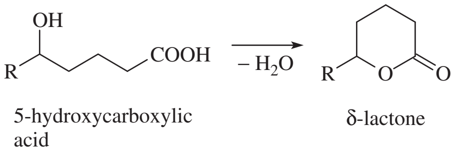


Figure 8.46 Dehydration of δ-hydroxycarboxylic acids.

Hydroxycarboxylic acid with the hydroxyl groups at distant carbons in the molecule, such as ricinoleic acid and related hydroxy fatty acids, yield internal esters called **estolides** upon heating. Estolides have also been found in oxidised lipids (see Section 3.8.1.14).

Malic acid dehydrates to form maleic acid when heated. During wine aging, tartaric acid yields 2,3-dihydroxymaleinic, (*S*)-2-hydroxy-3-oxobutanedioic, 2,3-dioxobutanedioic, formylglyoxylic (the precursor of glyoxal) and 2-hydroxymalonic (tartronic) acids (Figure 8.47). Heating of tartaric acid to temperatures of about 150 °C yields metatartaric acid lactide (see **11-39**). At higher temperatures, dehydration and isomerisation (as with β-hydroxy acids) leads to the formation of an intermediate oxosuccinic acid (Figure 8.48), decarboxylation of which (it behaves as α-hydroxy acid) yields pyruvic acid. Citric acid at temperatures around 150 °C (e.g. during roasting coffee or when it is used as a synergist in oil deodorisation) decomposes to form anhydrides, which react with water to form aconitic, itaconic and citraconic acids (Figure 8.49). Isomerisation of itaconic acid gives rise to citraconic acid.

Reactions of oxocarboxylic acids

Thermal degradation of 2-oxocarboxylic acids (α-ketocarboxylic acids) proceeds with elimination of carbon monoxide (decarbonylation, Figure 8.50) and the formation of the corresponding carboxylic acids. Heating of 3-oxoacids (β-keto acids) leads to decarboxylation and the formation of alkyl methyl ketones (Figure 8.51). Higher keto acids, such as γ-keto acids, partly isomerise by heating to the corresponding unsaturated hydroxy acids that form lactones by dehydration. For example, laevulinic acid yields two isomeric lactones, α-angelica lactone (see **8-101**, later) and β-angelica lactone (see **8-102**, later).

Reactions of aromatic carboxylic acids

Phenolic acids are precursors of a number of simple phenols, which result from the activities of microorganisms or during thermal processes. The main products of thermal degradation of cinnamic acids are 4-vinyl phenols that arise as decarboxylation products. The subsequent reactions yield the corresponding 4-formyl phenols, 4-ethyl phenols, 4-(prop-2-en-1-yl) phenols, 4-acetyl phenols

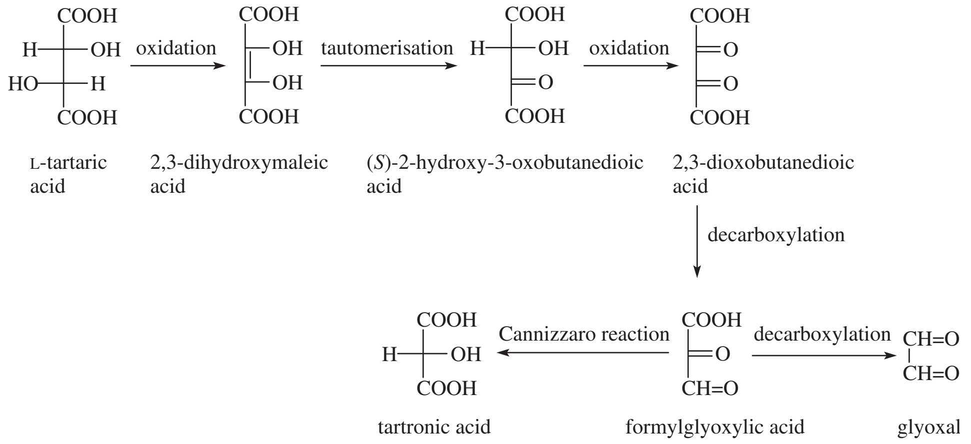


Figure 8.47 Reactions of tartaric acid during wine aging.

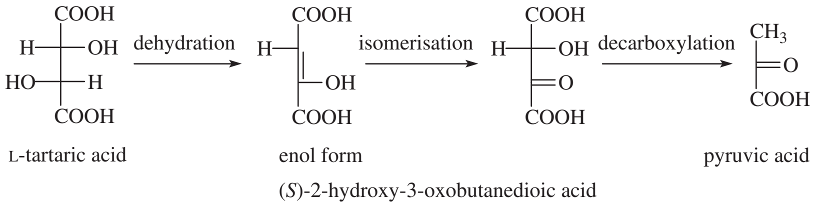


Figure 8.48 Thermal degradation of tartaric acid.

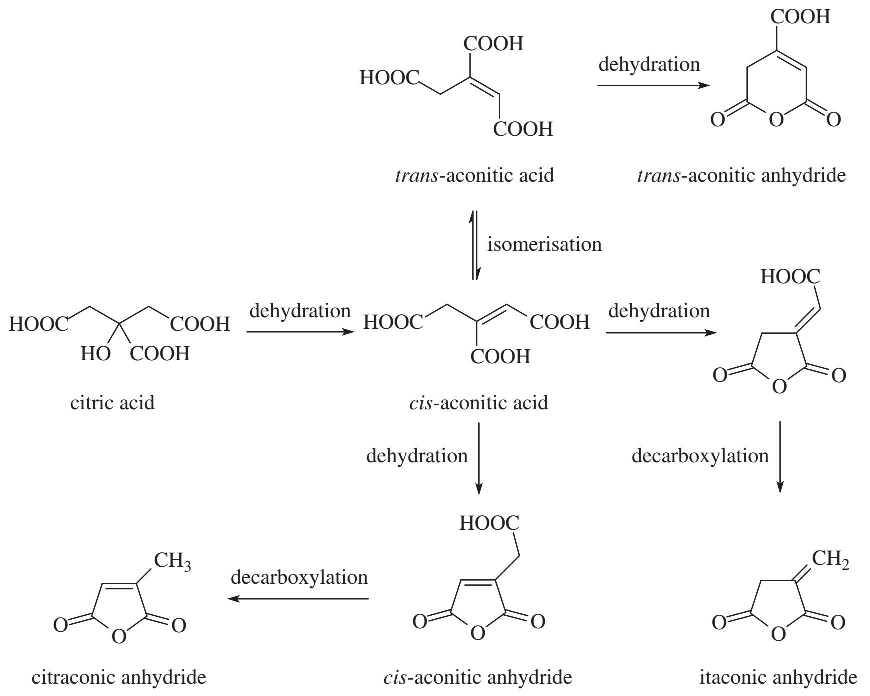


Figure 8.49 Thermal degradation of citric acid.

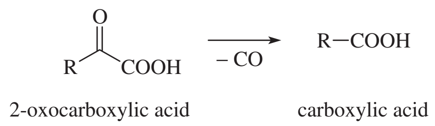


Figure 8.50 Thermal degradation of α-ketocarboxylic acids.

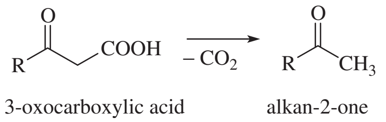


Figure 8.51 Thermal degradation of β-ketocarboxylic acids.

and many other compounds. Oxidation of phenolic acids and formation of condensation products are described in Section 9.12.4.

8.2.7 Functional derivatives of carboxylic acids

Numerous functional derivatives of carboxylic and substituted carboxylic acids have been found to be natural food components. In particular, important compounds are esters, lactones, anhydrides, amides and nitriles. The most important functional derivatives of carboxylic acids that act as odour-active substances are esters and lactones.

8.2.7.1 Classification, structure, nomenclature and occurrence

8.2.7.1.1 Esters

Volatile esters of aromatic carboxylic acids are often important components of plant floral scents, spices, fruits and vegetables. More than 1000 different compounds have been identified. In the flowers of plants, esters serve as signalling molecules, attractants, repellents and act in various defence mechanisms.

Numerous non-volatile esters derived from cinnamic acids are present in fruits, cereals, flowers, medicinal herbs, spices, fruits and vegetables. For example, common esters in grapes are diethyl malate and diethyl tartrate and depsides of tartaric acid with phenolic acids. An important group of non-volatile esters occurring in many plant materials are phenolic acid depsides with quinic, shikimic, malic and tartaric acids. For example, in coffee, esters of (*E*)-cinnamic acids with L-quinic acid, known as chlorogenic acid, occur. Most of these esters have virtually no effect on the odour of foods. However, they can influence the taste and colour of the foods, either by themselves or through some of their reaction products. For example, chlorogenic acid contributes to the bitter taste of coffee and has antioxidant, hypoglycaemic, antiviral, hepatoprotective and immunoprotective effects.

Esters of higher fatty acids are classified either as lipids or lipid accompanying substances. Some of these (esters of higher fatty acids with lower aliphatic alcohols, such as ethanol) are odour-active constituents found mainly in alcoholic beverages, but they usually only affect the taste or are flavour-indifferent substances.

Certain esters of higher fatty acids (e.g. esters of xanthophylls) are important colouring substances in foods of plant origin.

Volatile esters

Esters, reaction products of carboxylic acids with alcohols of general formula R^1-COOR^2, belong to the most widely occurring compounds in foods. They are often accompanied by the corresponding carboxylic acids and alcohols. The odour-active esters are mostly common esters of monocarboxylic acids, while esters of polycarboxylic acids are found less frequently. Alcohols bound in esters are either monohydric alcohols (containing only one hydroxyl group in each molecule) or polyhydric alcohols. Esters of lower aliphatic acids with lower aliphatic and aromatic alcohols are common odour-active compounds. They are especially important components of the primary flavour of fruits, vegetables, beverages and various spices. These esters arise by alcoholysis of acyl coenzymes A:

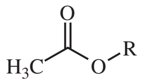
$$R^1\text{–CO–SCoA} + R^2\text{–OH} \rightarrow R^1\text{–CO–O–}R^2 + \text{HS–CoA}$$

For example, acetyl-CoA produces ethyl acetate by ethanolysis. In disrupted plant tissues, for example during production of juices, esters are rapidly broken down by various hydrolases, which results in a change of the flavour character. Also, many esters of aromatic acids are components of the aroma of fruits and spices.

During heating and long storage of foods, esters may also be formed in small quantities as secondary odour-active compounds. For example, esters are formed during aging of wines and spirits by esterification of carboxylic acids with alcohols (ethanol or fusel oil alcohols, but the non-enzymatic esterification is a very slow reaction), acidolysis (reaction of acids with esters), alcoholysis (reaction of alcohols with esters) or ester exchange (reaction of esters with other esters).

The lower fatty acid most frequently bound in esters is acetic acid, while formic, propionic, butyric and isobutyric acids occur less often. The common alcohol bound in esters is ethanol. However, esters of methanol, allyl alcohol, butan-1-ol; higher alcohols and very often esters of monoterpenic and aromatic alcohols also occur in foods, and esters of sulfur-containing alcohols are also common. Esters of low molecular weight acids and alcohols usually have a fruity odour; esters of terpenic alcohols with low molecular weight acids tend to have fragrant odours resembling flowers. Esters of aromatic acids and aromatic alcohols generally have heavy balsamic odours.

The most common ester of alcoholic beverages is ethyl acetate (**8-79**). In beer, for example, ethyl acetate concentrations range from 25 to 33 mg/l and in wines from 11 to 261 mg/l. Its threshold concentration is 150–170 mg/l; therefore ethyl acetate in wines very often acts as an odour-active component. Ethyl acetate in spirits (such as whisky) is present at levels of 45–460 mg/l.



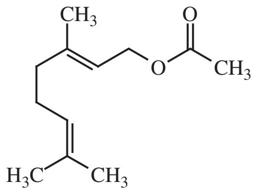
8-79, acetic acid esters (acetates)

ethyl acetate, R = CH_2CH_3
isobutyl acetate, R = $CH_2CH(CH_3)_2$
isopentyl acetate, R = $CH_2CH_2CH(CH_3)_2$

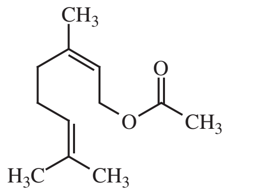
Other esters present in high concentrations in alcoholic beverages include isoamyl acetate and isobutyl acetate (**8-79**) that arise, as well as ethyl acetate, by alcoholysis of acetyl-CoA through the respective alcohols. Isobutyric and isovaleric acids yield virtually no esters in beer and wine, as the non-enzymatic esterification is too slow and the equilibrium is shifted to the detriment of esters. In spirits, these esters occur at levels up to units of mg/l.

Fatty acids bound in esters tend to have an even number of carbon atoms. These esters belong to the most aromatic substances influencing the bouquet of wines. For example, they are an important carrier of the fine flavour of Riesling wines and occur in larger quantities in heavy aromatic varieties such as Traminer, Muscat Ottonel and Sauvignon. The content of the main esters in wines is presented in Table 8.22. The ester content of musts is about ten times lower. Some sulfur-containing esters are also characteristic aromatic constituents of wines. For example, 3-mercaptohexyl acetate, produced by the fermentation of 3-mercaptohexan-1-ol (**8-15**), may occur in some wines (such as Traminer and Riesling) at a level of 0.5 μg/l, which is perceived as a fruity flavour.

Terpenoid esters occur in various fruits and many spices. Examples are the fairly widespread geranyl (**8-80**) and neryl acetates (**8-81**), which are components of citrus oils and odours of some spices (such as coriander).



8-80, geranyl acetate



8-81, neryl acetate

Table 8.22 Content of main esters in wines.

Ester[a]	Content (mg/kg)	Ester[a]	Content (mg/kg)
Ethyl acetate	11–261	Ethyl butyrate	0.2–4
Isobutyl acetate	0.1–11	Ethyl capronate	0.2–9
Isoamyl acetate	0.9–12	Ethyl caprylate	0.3–9
Hexyl acetate	0–9	Ethyl caprinate	0.1–9
Fenylethyl acetate	0–9	Ethyl lactate	12–378

[a] Composition refers to wines from the common grape vine *Vitis vinifera* (Vitiaceae). The natural component of grapes and wines from fox grape (*V. labrusca*) and some of its hybrids, originating in North America, is methyl anthranilate and ethyl anthranilate. In wines, these esters occur at levels up to 3.1 mg/l. Along with 2-aminoacetophenone formed by degradation of tryptophan, these esters may cause the characteristic unpleasant odour.

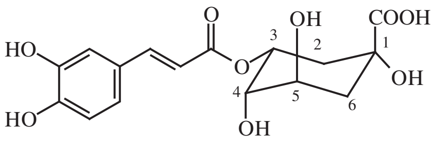
Non-volatile esters

The main aromatic carboxylic acids of coffee are **chlorogenic acids**, esters (depsides) of L-quinic acid with predominantly (*E*)-cinnamic acid, caffeic, ferulic and 4-coumaric acids. In smaller amounts, chlorogenic acids are found in tea, cocoa, apples and pears, in other fruits and in vegetables and potatoes.

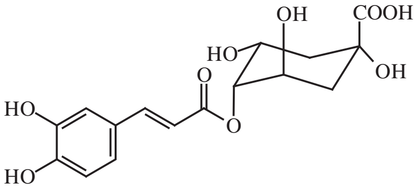
Green and roasted coffees, for example, contain more than 50 esters of cinnamic acids. Their level in green coffee beans ranges from 4 to 14%. The main groups are caffeoylquinic, dicaffeoylquinic, feruloylquinic, *p*-coumaroylquinic and caffeoyl-feruloylquinic acids. Moreover, all esters and diesters of quinic acid are found in three types of positional isomers that include 3-*O*-esters, 4-*O*-esters and 5-*O*-esters. The main component is chlorogenic acid, that is 3-*O*-caffeoyl-L-quinic acid, but this name also refers to all the natural quinic acid esters. The structures of the basic esters and diesters of caffeoylquinic acids are given in formulae **8-82**.

Numerous minor chlorogenic acids have recently been identified in coffee, which include diferuloylquinic, di-*p*-coumaroylquinic, dimethoxycinnamoylquinic and other chlorogenic acids, such as 3,4-di-*p*-coumaroylquinic, 3,5-di-*p*-coumaroylquinic and 4,5-di-*p*-coumaroylquinic acids; 3-*p*-coumaroyl-4-caffeoylquinic, 3-*p*-coumaroyl-5-caffeoylquinic, 4-*p*-coumaroyl-5-caffeoylquinic, 3-caffeoyl-4-*p*-coumaroylquinic, 3-caffeoyl-5-*p*-coumaroylquinic and 4-caffeoyl-5-*p*-coumaroylquinic acids; 3-*p*-coumaroyl-4-feruloylquinic, 3-*p*-coumaroyl-5-feruloylquinic and 4-*p*-coumaroyl-5-feruloylquinic acid; and 4-dimethoxycinnamoyl-5-*p*-coumaroylquinic acid. The minor chlorogenic acids amount to less than 1% of all chlorogenic acids.

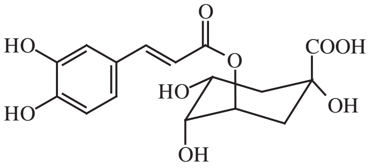
The chlorogenic acids content in roasted coffee and coffee beverages depend on the type and method of roasting the coffee seeds. Of about 80 varieties of three coffee trees, namely Arabic coffee (*Coffea arabica*, Rubiaceae), robusta coffee (*C. canephora*) and Liberian coffee (*C. liberica*), only the first two species are of commercial importance. *C. arabica* (the most common varieties are *typica*, *bourbon*, *mocha* and *maragogips*) represents about 75%, *C. canephora* (varieties *robusta*, *typical* and others) represents about 25% and *C. liberica* about 1% of world production. All varieties of *C. canephora* are commercially known by the name robusta. Their seeds have a higher content of chlorogenic acids than the seeds of *C. arabica*. Dicaffeoylquinic acids and mixed diesters



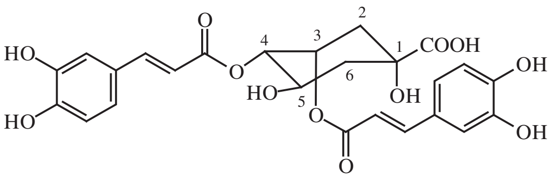
3-*O*-caffeoyl-L-quinic(chlorogenic) acid



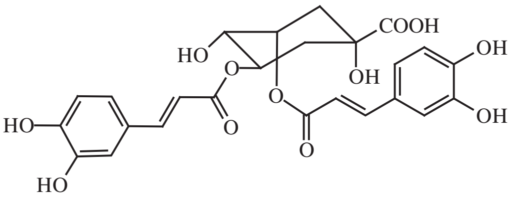
4-*O*-caffeoyl-L-quinic (cryptochlorogenic) acid



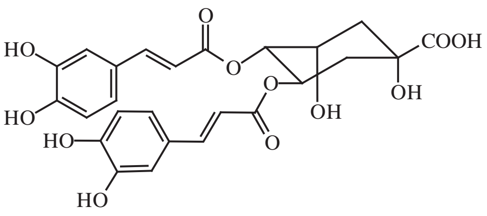
5-*O*-caffeoyl-L-quinic (neochlorogenic) acid



3,4-di-*O*-caffeoyl-L-quinic (isochlorogenic a) acid



3,5-di-*O*-caffeoyl-L-quinic (isochlorogenic b) acid



4,5-di-*O*-caffeoyl-L-quinic (isochlorogenic c) acid

8-82, chlorogenic acids

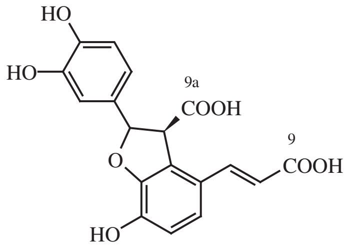
Table 8.23 Major hydroxycinnamic acids contents in roasted coffee (*Coffea arabica*).

Acid	Content (mg/kg)	Acid	Content (mg/kg)
3-Caffeoylquinic (chlorogenic)	20	3,4-Dicaffeoylquinic (isochlorogenic a)	0.1
4-Caffeoylquinic (cryptochlorogenic)	2	3,5-Dicaffeoylquinic (isochlorogenic b)	0.9
5-Caffeoylquinic (neochlorogenic)	1	4,5-Dicaffeoylquinic (isochlorogenic c)	0.1

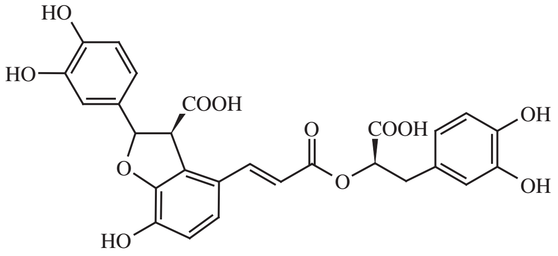
(caffeoyl-feruloyl quinic acids) and derivatives of some amino acids (such as *N*-caffeoyl-L-tryptophan and *N*-caffeoyl-L-tyrosine) are found only in the robusta coffee.

During roasting of coffee seeds, about 30–70% chlorogenic acids are transformed into γ-lactones of 3-*O*- and 4-*O*-cinnamoylquinic acids (cinnamoyl-1,5-quinolactones) and other products. These lactones are the most important bitter substances of roasted coffee. The main component is 3-*O*-caffeoyl-L-quino-1,5-lactone, also known as 3-*O*-caffeoyl-γ-quinide (see Section 8.3.5.1.4). The levels of chlorogenic acids in roasted coffee are given in Table 8.23. The chlorogenic acids contents in coffee surrogates, for example in roasted chicory root, are lower than in roasted coffee seeds. Raw potatoes contain 100–200 mg/kg of chlorogenic acids, their content in boiled potatoes is about 35% of this amount, and the content in baked potatoes is almost negligible.

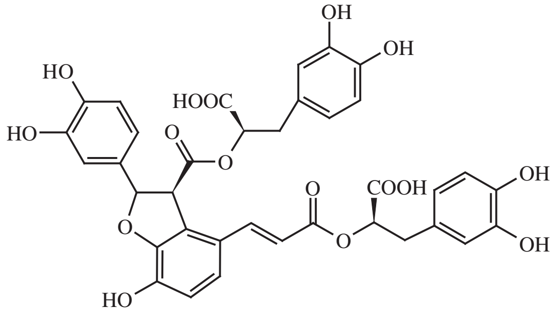
Some other caffeic acid esters found in aromatic and medicinal plants have significant antioxidant and other biological effects. An example is caffeic acid ester with (+)-(*R*)-3,4-dihydroxyphenyllactic acid known as rosmarinic acid (**8-83**), which is an important antioxidant distributed mainly in plants of the mint family (*Lamiaceae*). These plants include rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*). For example, rosmarinic acid and more than 20 caffeic acid derivatives, including various oligomers, have been isolated from red sage, also known as Chinese sage (*Salvia miltiorrhiza*), the root of which is used in traditional Chinese medicine against coronary and cerebrovascular diseases, various types of hepatitis and chronic renal disease. Examples of oligomers of the dihydrobenzofuran type is a dimer called prolithospermic acid (**8-84**), lithospermic acid (**8-85**) (a trimer), and lithospermic acid B, also known as salvianolic acid B (**8-86**), which is a tetramer. In lithospermic acid and lithospermic acid B, the C-9 (and respectively C-9a) carboxyl of prolithospermic acid is esterified with 3,4-dihydroxyphenyllactic acid. The plant also contains a number of diterpenoids with antioxidant effects.



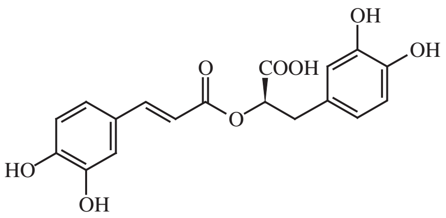
8-84, prolithospermic acid



8-85, lithospermic acid



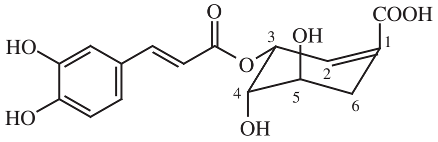
8-86, lithospermic acid B



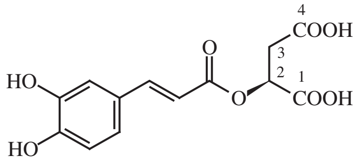
8-83, rosmarinic acid

A representative of shikimic acid depsides is 3-*O*-caffeoyl-L-shikimic acid, known as dactyliferic acid (**8-87**), which occurs in dates. This acid and other related depsides are substrates of oxidoreductases in enzymatic browning reactions in dates. The seeds and shoots of plants of the genus *Brassica* of the cabbage family (Brassicaceae) contain various esters of cinnamic acids with malic

acid, such as 2-*O*-(4-coumaroyl)-L-malic, 2-*O*-caffeoyl-L-malic (**8-88**), 2-*O*-feruloyl-L-malic and 2-*O*-sinapoyl-L-malic acids.

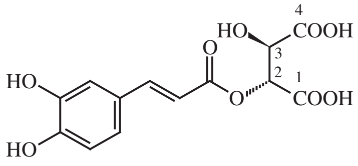


8-87, 3-*O*-caffeoyl-D-shikimic acid

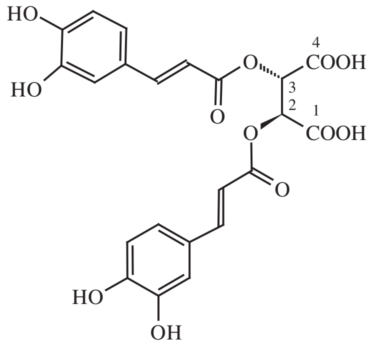


8-88, 2-*O*-caffeoyl-L-malic acid

Grape musts and wines contain depsides of some phenolic acids with L-tartaric acid. Common esters are depsides of caffeic (**8-89**), 4-coumaric and vanillic acids. For example, the vanilloyltartaric acid content in musts and wines ranges from 1.4 to 11.7 mg/l, and of 4-coumaroyltartaric (cutaric) acid and caffeoyltartaric (caftaric) acid is 0.6–5.5 and 10.2–26.9 mg/l, respectively. In all these depsides, (*E*)-isomers dominate. 2,3-Di-*O*-caffeoyl-L-tartaric acid, known as cichoric acid (**8-90**), is an example of diesters of L-tartaric acid, which occur in plants of the Asteraceae family. Cichoric acid is situated in the root and leaves of chicory (*Cichorium intybus*), endive (*C. endivia*) and lettuce (*Lactuca sativa*).

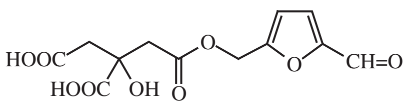


8-89, 2-*O*-caffeoyl-L-tartaric acid

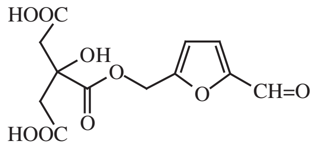


8-90, 2,3-di-*O*-caffeoyl-L-tartaric acid

Fruit-juice concentrate of Japanese apricot of the *Armeniaca* section of the genus *Prunus* (*P. mume*), which is effective against influenza A infection, contains, as the active component, esters of 5-hydroxymethyl-2-furancarbaldehyde with citric (**8-91**) and malic acids (**8-92**).

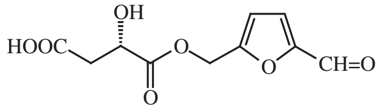


1-[5-(2-formylfuryl)methyl]dihydrogen
2-hydroxypropane-1,2,3-tricarboxylate (mumefural)

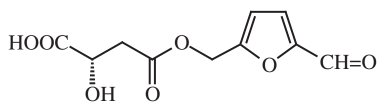


2-[5-(2-formylfuryl)methyl]dihydrogen
2-hydroxypropane-1,2,3-tricarboxylate

8-91, esters of 5-hydroxymethylfuran-2-carbaldehyde with citric acid



1-[5-(2-formylfuryl)methyl]hydrogen 1-hydroxyethane-1,2-dicarboxylate



2-[5-(2-formylfuryl)methyl]hydrogen 1-hydroxyethane-1,2-dicarboxylate

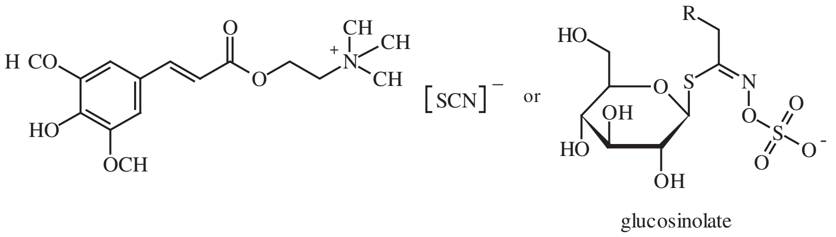
8-92, esters of 5-hydroxymethylfuran-2-carbaldehyde with malic acid

Esters of sinapic acid, such as *O*-sinapoyl choline (**8-93**) and sinapoyl malate (2-*O*-sinapoyl-L-malic acid; **8-94**), are found primarily in cruciferous plants (Brassicaceae), where they belong to the main phenylpropanoids accumulated. Their counter ions are normally thiocyanate ions ($S{=}C{=}N^- \leftrightarrow {}^-S{-}C{\equiv}N$) and in the intact seeds, glucosinolates. Sinapine-glucoraphanin salt (see Table 10.13) has recently been isolated from broccoli seeds. Sinapoyl choline is found exclusively in plant seeds, which means that it occurs also in the seeds of oilseed rape (rape seed, *Brassica napus*) and turnip rape (*B. rapa*, syn. *B. campestris*), used as a source of rapeseed oil. The seeds contain up to 1% sinapoyl choline, known as sinapine. In sinapine both sinapic acid isomers occur, but the (*E*)-isomer always prevails (**8-93**). The term sinapines is used generally for all choline esters with phenolic acids. The composition of phenolic acids in rapeseed flour is shown in Table 8.24. About 85% of phenolic acids are bound in sinapines, while the rest are free acids or their glycosides. 4-Hydroxybenzoic acid, rather than sinapic acid, prevails in the white mustard seeds (*Leucosinapis alba*, syn. *Brassica alba*) that are used raw or roasted in dishes, and mixed with other ingredients are used to produce a mustard paste or sauce that is used as a condiment.

In germinating seeds, the choline esters are transformed into the corresponding malates. In young plants 2-*O*-sinapoyl-L-malic acid predominates (**8-94**), while the main depsides in older plants are 2-*O*-(4-coumaroyl)-L-malic acid and 2-*O*-caffeoyl-L-

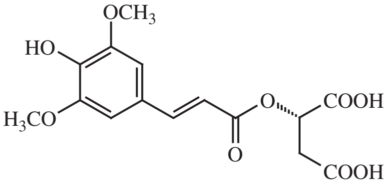
Table 8.24 Phenolic acids in rapeseed flour.

	Content (mg/kg)			Content (mg/kg)	
Acid	Free acids	Esters	Acid	Free acids	Esters
Salicylic	1-31	7-10	*o*-Coumaric	3-11	-
Gentisic	Traces-8	Traces-9	*p*-Coumaric	Traces-30	Traces-8
4-Hydroxybenzoic	0-22	Traces-27	(*E*)-Caffeic	Traces-18	0-Traces
Protocatechuic	4-14	Traces-18	(*Z*)-Caffeic	-	Traces-21
Vanillic	Traces-9	Traces-12	(*E*)-Ferulic	9-47	8-79
Syringic	1-24	Traces-23	(*E*)-Sinapic	35-516	1713-5971
Cinnamic	Traces-10	-	(*Z*)-Sinapic	32-101	445-989



8-93, (*E*)-*O*-sinapoyl choline thiocyanate and its counter ions

malic acid. Sinapoyl malate protects plants against UV-B radiation (280–320 nm); the function of sinapoyl choline is still mainly unknown.

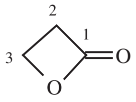


8-94, 2-*O*-sinapoyl-L-malic acid

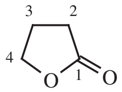
Sinapoyl esters are considered antinutritional compounds because they have a bitter and astringent taste, thus contributing to the bitter taste of rapeseed meal. The intensity of the bitter taste is comparable to the intensity of the bitter taste of caffeine. In the refining of rapeseed oil, sinapines form complexes with proteins. They show lower antioxidant activity than the corresponding phenolic acids and do not have antimicrobial effects. Sinapines present in the feed of some breeds of laying hens cause an off-flavour and fish-like odour of yolks.

8.2.7.1.2 Lactones

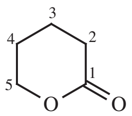
Lactones are internal esters of hydroxycarboxylic acids. They are characterised by the number of carbon atoms (methylene groups) present between the carboxyl group and carbon to which is bound the hydroxyl group. β-Lactones ($n = 1$) (**8-95**), γ-lactones ($n = 2$) (**8-96**) and δ-lactones ($n = 3$) (**8-97**) have been identified. γ-Lactones and δ-lactones are formed by dehydration of the hydroxy acids. Dehydration of β-hydroxy acids, however, does not produce β-lactones, but gives rise to α,β-unsaturated carboxylic acids.



8-95, β-propiolactone



8-96, γ-butyrolactone

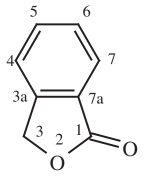


8-97, δ-valerolactone

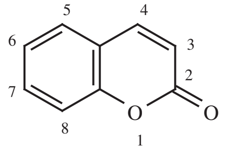
Nomenclature of lactones is based on the nomenclature of hydroxycarboxylic acids. The simplest β-lactone hypothetically derived from β-hydroxycarboxylic acids is propano-3-lactone (formerly also known as 3-propanolide and β-propiolactone, **8-95**), which can be considered an internal ester of 3-hydroxypropanoic acid. The simplest γ-lactone is butano-4-lactone, also known as 4-butanolide or γ-butyrolactone (**8-96**), which is derived from 4-hydroxybutanoic acid. The basic member of the homologous series of δ-lactones is pentano-5-lactone, also known as 5-pentanolide or δ-valerolactone (**8-97**), which is derived from

5-hydroxypentanoic acid. Lactones can also be considered as oxygen heterocyclic compounds. β-Lactones are actually oxetanes, γ-lactones are oxolanes or tetrahydrofuranes and δ-lactones are oxanes or tetrahydropyranes.

Lactones occur as natural odorants in all major food commodities, including meat and meat products, milk, dairy products, cereals, fruits, vegetables and various beverages, such as tea, wine and spirits. Odour-active compounds in foods are γ- and δ-lactones derived from aliphatic saturated and unsaturated γ-hydroxycarboxylic and δ-hydroxycarboxylic acids derived from fatty acids or sugars, but some lactones also arise from other precursors (e.g. mint lactone is a terpenoid compound and pantolactone is produced by hydrolysis of pantothenic acid via pantoic acid). Some lactones derived from aromatic hydroxycarboxylic acids are also common compounds. The most important representatives of these compounds are phthalides that are 3*H*-isobenzofuran-1-ones (**8-98**) and coumarins (**8-99**) that are 2*H*-1-benzopyran-2-ones (δ-lactones of 2-hydroxycinnamic acids).



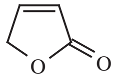
8-98, phthalide



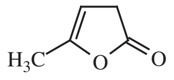
8-99, coumarin

Lactones of aliphatic hydroxycarboxylic acids

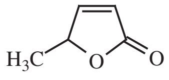
The simplest lactones are γ-butyrolactone (butane-4-lactone, **8-96**) and γ-crotonolactone (but-2-eno-4-lactone, **8-100**). These compounds are found as common constituents of many foods in small amounts. γ-Butyrolactone has a faintly sweet odour reminiscent of rancid butter, and the smell of γ-crotonolactone is like rancid fat. The methyl derivatives pent-3-eno-4-lactone (α-angelica lactone, **8-101**) and pent-2-eno-4-lactone (β-angelica lactone, **8-102**) are produced as degradation products of hexoses in acid solutions via laevulinic acid and have a sweet, herbal odour.



8-100, γ-crotonolactone

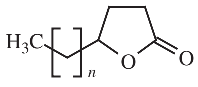


8-101, α-angelica lactone



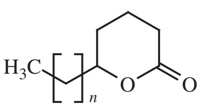
8-102, β-angelica lactone

γ-Lactones (**8-103**) and δ-lactones (**8-104**) of some aliphatic straight chain hydroxycarboxylic acids are flavour-significant components of fruits and dairy products. For example, the odour of γ-nonalactone (nonano-4-lactone) is reminiscent of coconut. γ-Decalactone (decano-4-lactone) has a fruity odour reminiscent of peaches, and is a component of apricots and strawberries. δ-Decalactone (decano-5-lactone), the odour of which resembles peaches, is a component of raspberries and coconuts. γ-Undecalactone (undecano-4-lactone) has an intense peach-like odour, and the odour of γ-dodecalactone (dodecano-4-lactone) is reminiscent of peaches with butter notes. δ-Dodecalactone (dodecano-5-lactone) and γ-decalactone are the major carriers of flavour of condensed milk and heated milk fat (butter). Dishes prepared with butter then have a typical odour reminiscent of clarified butter. Some lactones, such as γ-undecalactone and δ-dodecalactone are used for flavouring dairy products and margarines. All these lactones exist as a pair of enantiomers, the abundance of which varies greatly, but the dominating forms are (+)-(*R*)-stereoisomers. For example, coconut oil contains 75% of the (*R*)-isomer of δ-decalactone and 25% of the desired (*S*)-isomer (**8-105**), the content of (*R*)-γ-dodecalactone in raspberries is about 50% of the total γ-dodecalactone concentration, while strawberries contain this enantiomer exclusively.



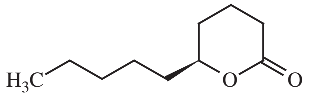
8-103,γ-lactones of saturated hydroxycarboxylic acid

nonano-4-lactone, *n* = 4
decano-4-lactone, *n* = 5
undecano-4-lactone, *n* = 6
dodecano-4-lactone, *n* = 7



8-104, δ-lactones of saturated hydroxycarboxylic acids

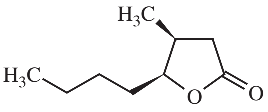
nonano-5-lactone, *n* = 3
decano-5-lactone, *n* = 4
undecano-5-lactone, *n* = 5
dodecano-5-lactone, *n* = 6



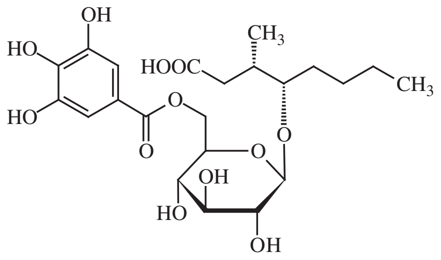
8-105, (*S*)-δ-decalactone

Other important odorous components of foods are fragrant lactones derived from branched aliphatic hydroxycarboxylic acids. The trivial names are normally preferred. An important component of the aroma of wines and spirits aged in oak barrels (such as whisky) is the so-called whisky lactone, 5-butyl-4-methyl-4,5-dihydro-3*H*-furan-2-one (3-methyloctano-4-lactone, 3-methyl-4-octanolide or 4-butyl-3-methylbutyrolactone), also known as cognac or oak lactone, which can exist in four stereoisomers, of which the (3*S*,4*S*)-isomer (**8-106**) predominates. In brandy, for example, the content of the (*Z*)-isomers, (3*R*,4*R*)- and (3*S*,4*S*)-isomers, that have a desirable flavour, ranges between 30 and 247 μg/l. The (*E*)-isomers, (3*S*,4*R*)- and (3*R*,4*S*)-isomers, are found at a concentration of 115–736 μg/l. The precursor of the (3*S*,4*S*)-isomer in oak wood

(*Quercus petraea*, Fagaceae) is 6′-*O*-gallate derivative of (3*S*,4*S*)-4-β-D-glucopyranosyloxy-3-methyloctanoic acid (**8-107**).

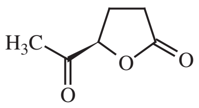


8-106, (3*S*,4*S*)-whisky lactone

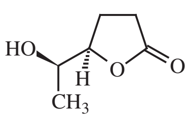


8-107, (3*S*,4*S*)-whisky lactone precursor in oak wood

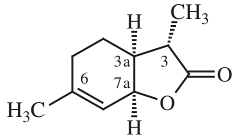
An important γ-lactone in wines is 5-oxohexano-4-lactone known as solerone, which recalls the smell of wine. It occurs as a mixture of (*R*)- (**8-108**) and (*S*)-enantiomers. The reduced form of solerone with a fruity odour, 4,5-dihydroxyhexano-4-lactone, is known under the trivial name of solerole or sherry lactone as it occurs in sherry-type wines, as a mixture of (4*R*,5*R*)- (**8-109**) and (4*S*,5*S*)-diastereoisomers. The almost racemic composition of solerone and solerole is attributed to racemisation during wine storage. An important lactone related to phthalides is 3*a*,4,5,7*a*-tetrahydro-3,6-dimethyl-3*H*-benzofuran-2-one, also known as 3*H*-benzofuran-2-one or wine lactone (**8-110**). Of the eight possible stereoisomers, only the (3*S*,3*aS*,7*aR*)-isomer with a lactonic, herbal and sweet odour is found in white wines in amounts of 0.07–0.2 μg/l, but the odour threshold concentration is very low (0.000 01-0.00 004 ng/l in air). Many foods contain 2-hydroxy-3-methylpent-2-eno-4-lactone, also known as 3-hydroxy-4,5-dimethyl-5*H*-furan-2-one or sotolon (the trade name). Sotolon is a key odorant of fenugreek (*Trigonella foenum-graecum*, Fabaceae), lovage and sake and occurs



8-108, (*R*)-solerone



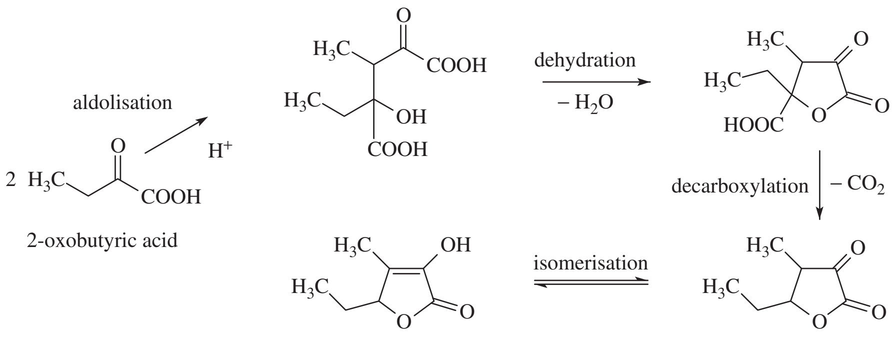
8-109, (4*R*,5*R*)-solerole



8-110, (3*S*,3a*S*,7a*R*)-wine lactone

as a flavouring component of meat, bread and roasted coffee, white wines, sherry, Madeira and Port type wines. Sotolon can result from a number of precursors. In acid protein hydrolysates, sotolon may arise from pyruvic and 2-oxobutyric acids analogously to the formation of abhexon that is produced from two molecules of 2-oxobutyric acid (Figure 8.52). A parallel mechanism responsible for the low concentrations of sotolon found in prematurely aged white wines could be an aldol condensation between 2-oxobutyric acid and acetaldehyde. Another possibility is the formation from biacetyl and glycolaldehyde (Figure 8.53). The precursor of sotolon in fenugreek seeds and in the Maillard reaction is 3-amino-4,5-dimethyl-5*H*-furan-2-one. 3-Amino-4,5-dimethyl-5*H*-furan-2-one arises from pyruvic acid and formaldehyde (or glyoxylic acid and acetaldehyde) and any amino acid. Its hydrolysis yields sotolon (Figure 8.54).

Certain 2-hydroxy substituted γ-lactones (3-hydroxy furan-2-ones) derived from γ-crotonolactone, however, have a specific odour. Typically 3-hydroxy-4,5-dimethyl-5*H*-furan-2-one (sotolon) has an aroma resembling fenugreek or curry at higher concentrations, and caramel, maple syrup or burnt sugar at lower concentrations. Sotolon occurs as a mixture of (−)-(*R*)- and (+)-(*S*)-enantiomers (**8-111**). The perception threshold of (*S*)-sotolon (0.8 μg/l) in a model wine solution was 100 times lower than that of the (*R*)-enantiomer (89 μg/l), indicating that mainly (*S*)-sotolon contributes to the characteristic aroma of wines. The key component of acid protein hydrolysates is the higher



5-ethyl-3-hydroxy-4-methyl-5*H*-furan-3-one

Figure 8.52 Formation of lactones from 2-oxocarboxylic acids.

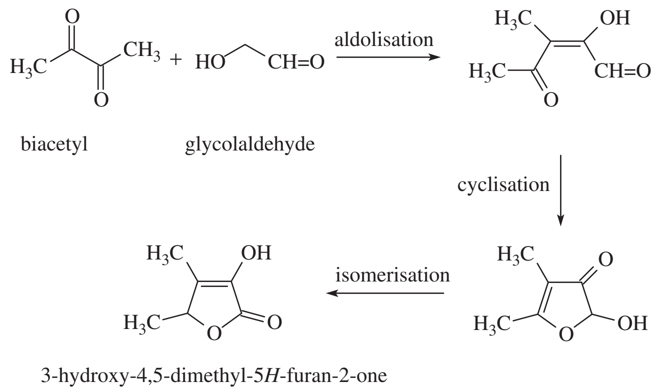


Figure 8.53 Formation of sotolon from biacetyl and glycolaldehyde.

homologue of sotolon, 5-ethyl-3-hydroxy-4-methyl-5*H*-furan-2-one, also known as maggi lactone, maple furanone or under the trade name abhexon. (−)-(*R*)-Abhexon (**8-112**) has an intense typical hydrolysate and maple syrup-like odour; (+)-(*S*)-abhexon has a less intense odour. In acid protein hydrolysates, abhexon is produced from reaction sequences analogous to those for sotolon (Figure 8.52), but from two molecules of 2-oxobutyric acid. Its content in acid protein hydrolysates is 1–4 mg/l.

8-111, (*R*)-sotolon **8-112**, (*R*)-abhexon

Dimethylsubstituted unsaturated γ-lactones that result from the autoxidation or photooxidation products of furan fatty acids are called bovolides. Bovolides have been found in some plants, butter, cooked meats and seafood. The especially important odour-active compounds are 3,4-dimethyl-5-pentylidene-5*H*-furan-2-one, called bovolide (Figure 8.55), and 3,4-dimethyl-5-pentyl-5*H*-furan-2-one that is known trivially as dihydrobovolide. Both compounds are characterised by their smell, which resembles celery. The mechanism of autoxidation of fatty acids is described in detail in Section 3.8.1.8.2.

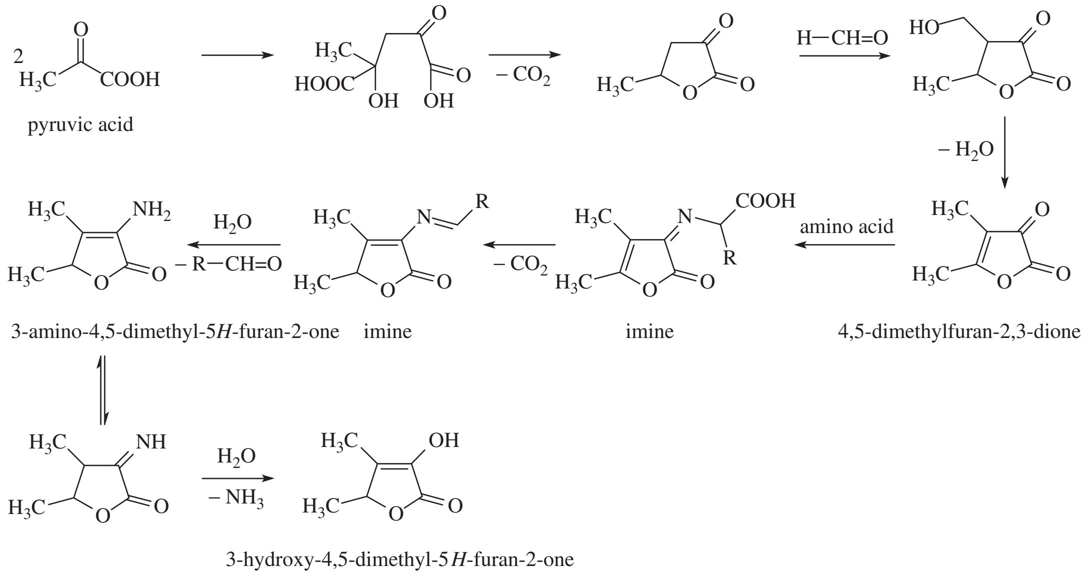


Figure 8.54 Formation of sotolon from pyruvic acid, formaldehyde and amino acids.

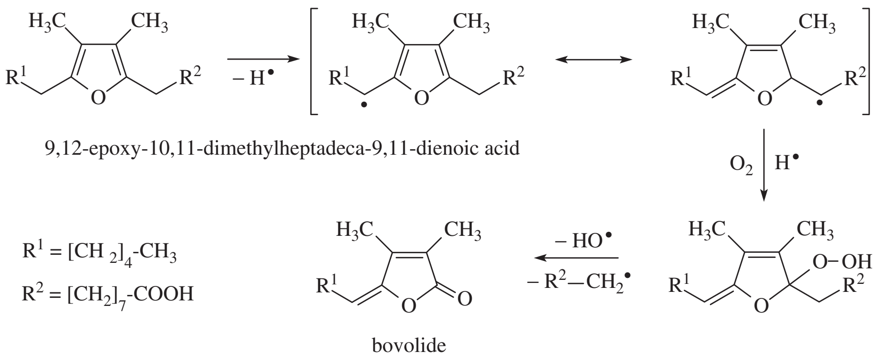
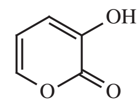
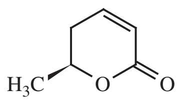


Figure 8.55 Formation of bovolide.

Important taste- and odour-active substances also include some δ-substituted lactones. 3-Hydroxypyran-2-one (3-hydroxy-2*H*-pyran-2-one), also known as isopyromucic acid (**8-113**) is the main degradation product of ascorbic and dehydroascorbic acids. Its aroma, which is reminiscent of liquorice and caramel, can be detected, for example, in dehydrated orange juices. The so-called parasorbic acid (**8-114**), that is (*S*)-2-methyl-2,3-dihydropyran-6-one or (*S*)-hex-2-en-5-olide, has certain biological (mutagenic and carcinogenic) effects. By heating in solutions, parasorbic acid yields sorbic acid via dehydration of 5-hydroxyhex-2-enoic acid that arises by opening the lactone ring. Parasorbic acid is found in rowan berries (*Sorbus aucuparia*, Rosaceae).



8-113, 3-hydroxy-2*H*-pyran-2-one

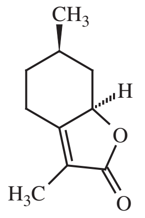


8-114, parasorbic acid

Other lactones

An example of terpenoid lactones that widely occur in plants is monoterpenic lactone (6*R*,7a*R*)-3,6-dimethyl-5,6,7,7a-tetrahydro-4*H*-1-benzofuran-2-one, known as mint lactone (**8-115**), with mint, herbaceous and coumarinic odours that is found together with related lactones in the essential oil of peppermint (*Mentha x piperita*, Lamiaceae), pennyroyal (*M. pulegium*) and other mint oils.

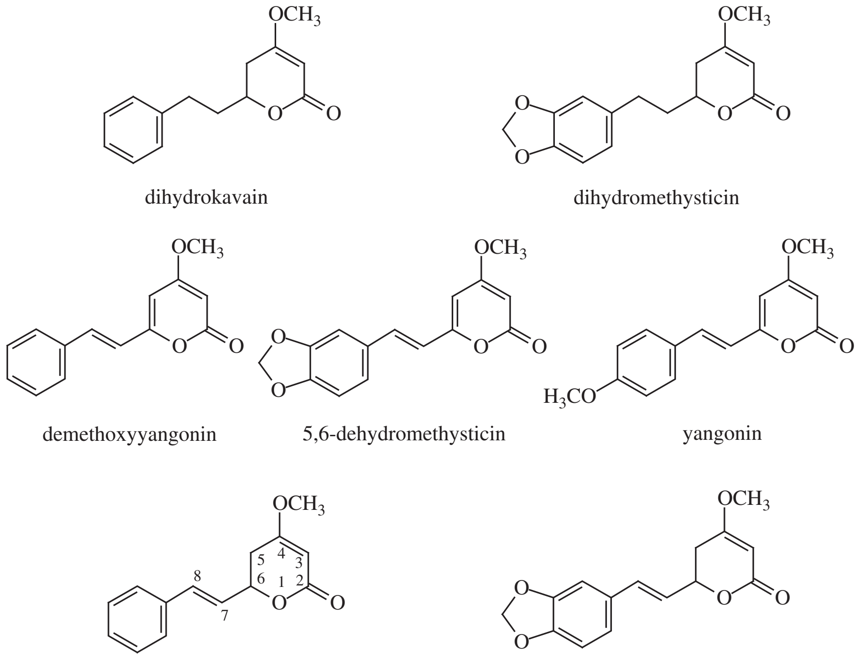
Typical polyketides related to lignans and flavonoids are styrylpyrones derived from cinnamic acids (the corresponding acyl-CoAs). An example of styrylpyrones is (2*R*,1′*E*)-4-methoxy-2-(2′-phenyleth-1′-en-1′-yl)-2,3-dihydropyran-6-one, better known as kavain or kawain and formerly as genosan (**8-116**). Kavain, along with more than 18 related compounds called kava pyrones (kava lactones), is present in kava-kava (*Piper methysticum*, Piperaceae) that grows on the islands of Micronesia and Polynesia. The other major constituents are methysticin, demethoxyyangonin, yangonin, dihydrokavain, dihydromethysticin and 5,6-dehydromethysticin (**8-116**), which belong to three different systems A–C. The A system is characterised by the absence of double bonds in both the positions 5,6 and 7,8; B is a completely unsaturated system; and C has a double bond in the 7,8 position. A drink possessing narcotic effects that is called kava or kava-kava is prepared from the rhizomes, which contain about 12% kava pyrones in the dry state. Phytochemicals based on kava-kava are sold worldwide for the treatment of nervous anxiety, tension and restlessness.



8-115, mint lactone

Phthalides

Phthalides are unique components of aromatic vegetables of the carrot family Apiaceae. They are a group of related γ-lactones, (3*H*)-isobenzofuran-1-ones. The benzene ring of phthalides may be partially or fully unsaturated (in hexahydrophthalides, tetrahydrohydrophthalides and dihydrohydrophthalides) and there can



dihydrokavain, dihydromethysticin, demethoxyyangonin, 5,6-dehydromethysticin, yangonin, kavain, methysticin

8-116, kava lactones

be alkyl or alkylidene substituents in position C-3 of the heterocyclic ring.

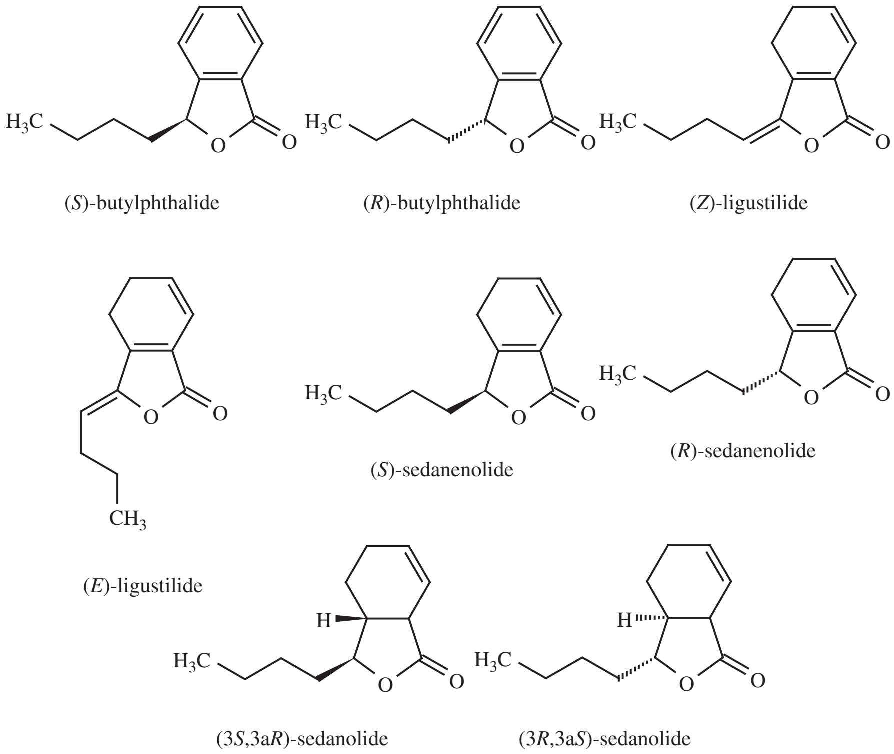
Biosynthesis of phthalides is associated with biosynthesis of styrylpyrones, as both types of compounds are ketides, but phthalides are derived from aliphatic carboxylic acids (the corresponding acyl-CoAs). Phthalides in plants have various regulatory functions at the cellular level. They exhibit spasmolytic, hypotensive, sedative and diuretic effects.

In particular, phthalides are found in celeriac (*Apium graveolens* var. *rapaceum*), celery (*A. g.* var. *dulce*) and lovage (*Levisticum officinale*). In smaller quantities, phthalides are present as aromatic components of garden parsley (*Petroselinum hortense*), dill (*Anethum graveolens*), coriander (*Coriandrum sativum*), fennel (*Phoeniculum vulgare*) and other highly aromatic and flavourful plants of the Apiaceae family that have culinary and medicinal uses. The main components present in celery are 3-butyl-4,5-dihydrophthalide (also known as sedanenolide or senkyunolide, **8-117**), 3-butyl-3a,4,5,6-tetrahydrophthalide (also known as sedanolide or neocnidilide), 3-butylphthalide and 3-butylidene-4,5-dihydrophthalide called (*Z*)-ligustilide. Other dihydro-, tetrahydro- and hexahydrophthalides are present in small quantities. The main fragrant component of lovage is (*Z*)-ligustilide and another important compound is sedanenolide. The main phthalides of the leaves and roots of parsley cultivars are sedanenolide, (*E*)-ligustilide and 3-butylphthalide, sedanolide is the main phthalide of dill and coriander.

Isomeric phthalides have an odour reminiscent of celery or acid protein hydrolysates, but the key component of protein hydrolysates is maggi lactone, known as abhexon. Phthalides occur as numerous isomers in varying amounts in various plants. For example, the content of 3-butylphthalide (with the celery stem-like odour typical of celery and celeriac) in tubers of celery is about 4 wt% and about 88% of this amount is the (*S*)-isomer and 12% the (*R*)-isomer, which has about 1000 times less intense flavour (0.01 μg/l). The amount of 3-butylphthalide in bulb-like stem bases of fennel is only about 0.01%, but the main isomer (64%) is (*R*)-3-butylphthalide. The odour of (−)-(*S*)-sedanenolide (odour threshold of 0.14 μg/l) resembles celery stem, while the odour of (+)-(*R*)-sedanenolide (odour threshold of 0.6 μg/l) is more celery leaf-like. The (−)-(3*S*,3a*R*)-sedanolide has natural celery leaf odour (odour threshold of 0.01 μg/l in 1% ethanol), while the (+)-(3*R*,3a*S*)-sedanolide has more celery seed odour with a weak hint of celery, but its odour threshold is higher (0.03 μg/l in 1% ethanol).

Coumarins

A special group of δ-lactones formally derived from the hydroxycinnamic acids are coumarins with a skeleton of 2*H*-benzopyran-2-one, which is also called chromen-2-one or 5,6-benzo-2-pyrone (**8-99**). More than 1000 coumarins are found in nature, but only the basic member of the homologous series of coumarins performs as an aromatic compound: this is called coumarin. Plant materials also contain a number of non-volatile coumarins substituted with hydroxyl and methoxyl groups and their glycosides. These and other coumarins, generally taking on the role of phytoalexins, such as isocoumarins, furanocoumarins and pyranocoumarins, are described in Section 10.3.2.5.2. Some isocoumarins are intensely sweet (such as phyllodulcin) or bitter substances (such as 6-methoxymellein),



8-117, phthalides

while 3-phenylcoumarins and 4-phenylcoumarins are coloured compounds that are classified as isoflavones and neoflavonoids, respectively. Most of the coumarins are biologically active substances that are normally classified as toxic food components.

Coumarin is found in many fresh plant tissues. Natural coumarin is obtained from the seeds of the tree *Dipteryx odorata* (syn. *Coumarona odorata*, Fabaceae), known as tonka beans, which is native to northern South America. The aromatic bark of an evergreen tree native to South Asia called Chinese cinnamon or cassia (*Cinnamomum aromaticum*, Lauraceae), which is used as a spice, contains high quantities of coumarin. European health agencies have warned against consuming large amounts of cassia, as the tolerable daily intake may be exceeded in consumers with a high intake of this spice containing high levels of coumarin (on average 2100–4400, but also up to 10 000 mg/kg). In larger quantities, coumarin occurs in the haulm of yellow sweet clover (yellow melilot; *Melilotus officinalis*, Fabaceae) and white sweet clover (white melilot; *M. albus*), sweet woodruff (*Galium odortum*, Rubiaceae) and some grass species, such as sweet grass (*Hierochloe odorata*, Poaceae). Coumarin is also found in small amounts in strawberries, apricots, liquorice rhizome (*Glycyrrhiza glabra*, Fabaceae) and other fruits and plant materials.

Plants only contain the β-D-glucoside of (*E*)-2-hydroxycinnamic (*o*-coumaric) acid, called melilotin or melilotoside (Figure 8.56) precursor of coumarin, which accumulates in the vacuoles along with the glucoside of (*Z*)-2-hydroxycinnamic (coumarinic) acid. Coumarin is only produced by plants during tissue injury when glucosides of coumaric and coumarinic acids come into contact with β-glucosidase. The glucoside of coumaric acid is isomerised to the glucoside of coumarinic acid by 2-coumarate β-D-glucoside isomerase, but the *trans–cis* isomerisation also occurs spontaneously by means of UV light. The last step is the spontaneous cyclisation (lactonisation) of coumarinic acid to coumarin, which can be further transformed into dihydrocoumarin and melilotic acid.

The coumarin scent vaguely recalls fresh clover and vanilla and is thus added to tobacco products, artificial vanilla substitutes in perfumes, cosmetic products and alcoholic beverages (such as Polish Zubrovka, which is flavoured with a stem of sweet grass). Coumarin was banned as a food additive in the 1950s because of its moderate toxicity (LD_{50} of 275 mg/kg) manifested by liver damage (hepatotoxicity) and kidneys damage (nephrotoxicity) and is on the list of substances of Regulation (EC) No. 1334/2008, which should not be added as such to foods as flavourings. The maximum level of naturally occurring coumarin in traditional and seasonal bakery products is restricted to 50 mg/kg and in fine bakery (containing a reference to cinnamon in the labelling) to 15 mg/kg, in breakfast cereals to 20 mg/kg and in desserts to 5 mg/kg. According to the German Federal Institute for Risk Assessment, its amount in food must not exceed the tolerable daily intake of 0.1 mg per kg body weight.

8.2.7.1.3 Nitriles

Only nitriles (**8-118**) are found in food; isomeric isonitriles (isocyanides) are not. Precursors of volatile nitriles are almost exclusively glucosinolates that occur in cruciferous plants of the family Brassicaceae. Nitriles (together with a number of other

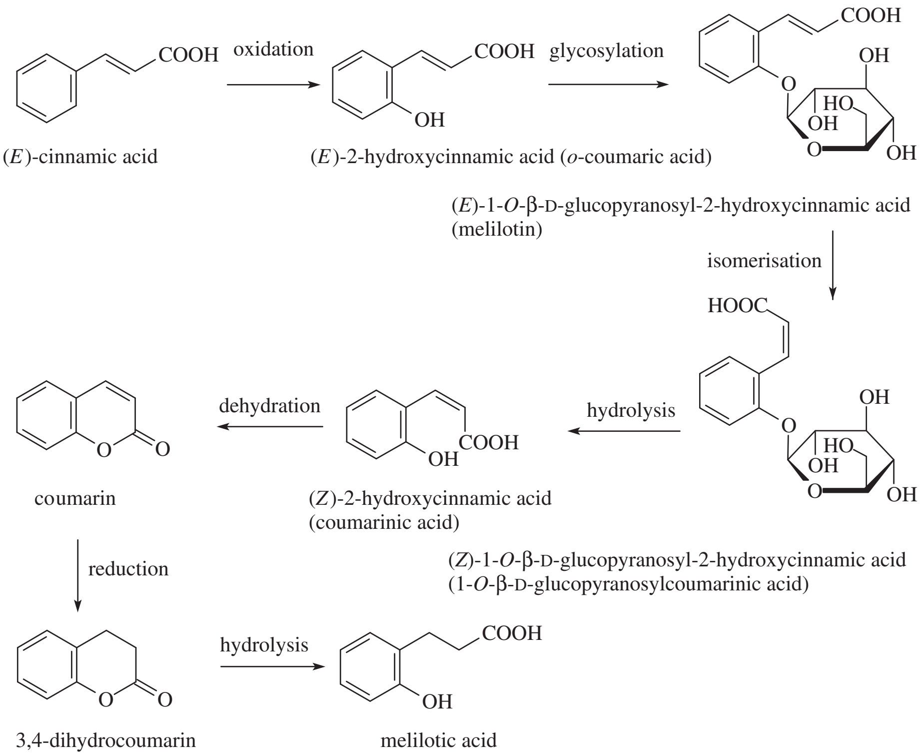


Figure 8.56 Biosynthesis of coumarin.

products) are formed by their enzymatic and thermal degradation. The main products are isothiocyanates (see Section 10.3.2.4.2).

8-118, nitriles

A common nitrile produced during processing of the vast majority of *Brassica* vegetables is but-3-enenitrile (allyl cyanide, **8-118**, R=CH=CH_2), which arises by degradation of glucosinolate sinigrin. For example, but-3-enenitrile acts, along with other compounds, as an odour-active compound in cooked cabbage and in sauerkraut. Other volatile nitriles include pent-4-enenitrile (but-3-en-1-yl cyanide, **8-118**, R=CH_2–CH=CH_2), which contributes considerably to the typical aroma of cabbage and some types of mustard pastes made from black and brown mustard seeds (*Brassica nigra* and *B. juncea*, respectively), and its higher homologue hex-5-enenitrile (pent-4-en-1-yl cyanide, **8-118**, R = CH_2CH_2CH=CH_2). These nitriles are formed from glucosinolates with aliphatic substituents known as gluconapin and glucobrassicanapin, respectively. Different cyanoepithioalkanes, glucosinolates with hydroxyalkenyl substituents, are formed as minor glucosinolate hydrolysis products, For example, progoitrin yields hydroxyalkenylnitriles and cyanohydroxyepithioalkanes.

An example of important non-volatile nitriles is 4-hydroxybenzyl cyanide, which is found in mustard pastes made from white mustard seeds (*Leucosinapis album*) containing glucosinolate sinalbin. Non-volatile nitriles also include cyanogenic glycosides, cyanogenic lipids and amino acids that contain the cyano group.

8.2.7.2 Properties and reactions

8.2.7.2.1 Esters

The organoleptic properties of some esters, which are important components of fruits, spices and fragrant flowers, are given in Table 8.25. One of the most important reactions of esters is hydrolysis (usually enzymatic hydrolysis), which usually has a negative effect on the flavour of many foods, especially fruits, where esters are important components of the primary flavour. Water is present in large excess in most foods, therefore heating or storage of foods usually leads to the hydrolysis of esters, even in the absence of hydrolytic enzymes. Chemical hydrolysis of esters is faster in alkaline solutions where salts of carboxylic acid are formed. Hydrolytic reactions of esters in foods are of great importance in materials rich in lipids and also in many other cases. For example, hydrolysis of pectins alters their ability to form gels and produces methanol.

In enzymatically inactive foods continual reactions proceed between acids, alcohols and esters. Owing to low reaction rates, these reactions never reach the equilibrium state. During heating or prolonged storage, less volatile acids bound in esters may be displaced by more volatile acids, by a process called acidolysis. A common example is the reaction of an ester with an alcohol, which is called alcoholysis. Alcoholysis helps improve the flavour of alcoholic beverages (especially fruit spirits) during long-term storage known as aging. One reason is the reaction of ethanol, which is present in large excess, with esters, the constituents of which are fusel oil alcohols.

8.2.7.2.2 Lactones

Generally, lactones with five-membered and six-membered rings are not too reactive. They are relatively stable in acidic solutions, where they typically arise by spontaneous dehydration of hydroxycarboxylic acids. In aqueous solutions, particularly in alkaline solutions, the lactone ring opens with the formation of a salt of the hydroxycarboxylic acid. The original lactone may result after acidification, especially on heating. Lactones react with alcohols to form esters and, similarly, the lactone ring opens on reaction with amino compounds to form amides.

8.2.7.2.3 Nitriles

Addition reactions to the nitrile group and hydrolysis of nitriles to the corresponding carboxylic acids may, to a certain extent,

Table 8.25 Organoleptic properties of important esters.

Ester	Odour character	Ester	Odour character
Allyl capronate	Pineapple	Ethyl lactate	Mandarin
Amyl acetate	Pear	Ethyl 2-methylbutyrate	Strawberries
Ammyl cinnamate	Cacao	Geranyl acetate	Rose
Ammyl formiate	Black currents, plum	(*Z*)-Hex-3-en-1-yl isovalerate	Rum
Amyl caprylate	Brandy, whisky	(*Z*)-Hex-3-en-1-yl butyrate	Apple
Butyl acetate	Pineapple	Isopulegyl acetate	Green apple
Butyl isobutyrate	Pineapple	Methylphenyl acetate	Mint
Butyl valerate	Apple	Methyl isovalerate	Honey
Cyclohexyl formiate	Sour cherry	Methyl capronate	Apple
Diethyl malonate	Apple	Methyl *N*-methylanthranilate	Pineapple

take place in foods containing nitriles. Nitriles occurring in trace amounts in rapeseed oils can be reduced to the corresponding amines during oil hydrogenation.

8.2.8 Phenols

8.2.8.1 Classification structure, terminology and occurrence

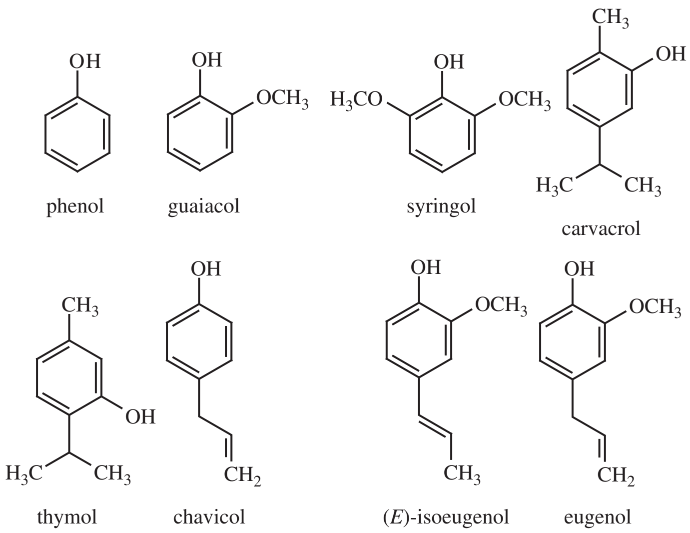
Phenols are components of virtually all foods. They are a very heterogeneous group of compounds, some of which may act also as odour-active compounds such as certain simple phenols formed as the degradation products of phenolic acids and products of their reduction, such as aldehydes and alcohols. Phenols are also important compounds influencing the taste of foods (simple phenols and polyphenols known as condensed tannins, called flavolans, that are responsible for astringent taste). Phenolic compounds include also many natural dyes (some quinones, lignans, flavonoids and related stilbenes, xanthones and other compounds). Particular phenols exhibit significant biological effects, and therefore act as the defensive substances of plants, called phytoalexins, as natural antioxidants and natural toxic compounds. These phenols are discussed elsewhere in this book. An overview of the basic groups of phenolic compounds occurring in foods is given in Table 8.26.

Phenols, which in foods act as aromatic substances, are either primary food components of some essential oils or are produced as secondary substances in food processing. The primary components are phenols structurally related to the corresponding alkyl aryl ethers (see Section 8.2.3.1.2). Secondarily formed phenols are produced mainly from phenolic acids and lignin during thermal processes and by the action of microorganisms. Particularly important compounds are derived from phenol, guaiacol (2-methoxyphenol) and syringol (2,6-dimethoxyphenol) (**8-119**).

Simple alkyl phenols derived from phenol, found as constituents of essential oils, include two major monoterpenes with a similar odour, carvacrol (5-isopropyl-2-methylphenol, **8-119**) and thymol (2-isopropyl-5-methylphenol, **8-119**). These phenols occur, for example, in the essential oil of thyme. Chavicol, also known as 4-prop-2-en-1-ylphenol or 4-allylphenol (**8-119**) is a component of the essential oil of basil. A number of other phenols are derived from guaiacol. Isoeugenol also occurs in the essential oil of basil as the (*E*)-isomer, (*E*)-2-methoxy-4-(prop-1-en-1-yl)phenol (**8-119**),

Table 8.26 Main types of food phenols.

Number of C atoms	Basic skeleton type	Groups of phenolic compounds
6	C_6	Simple phenols, benzoquinones
7	C_6-C_1	Benzoic acids
8	C_6-C_2	Acetophenones, phenylacetic acids
9	C_6-C_3	Phenylpropanoids, phenylpropenes, chromones, isochromenes, cinnamic acids, coumarins
10	C_6-C_4	Naphthoquinones
13	C_6-C_1-C_6	Xanthones
14	C_6-C_2-C_6	Anthraquinones, stilbenes, diarylethanoids
14	C_6-C_1-C_6-C_1, C_6-C_1-C_1-C_6, C_1-C_6-C_6-C_1	Hydrolysable tannins (dimers)
15	C_6-C_3-C_6	Flavonoids, pterocarpans, diarylpropanoids
16	C_6-C_4-C_6	Diarylbutanoids
16	C_6-C_{10}	Gingerols
17	C_6-C_5-C_6	Diarylpentanoids
18	C_6-C_3-C_3-C_6, C_6-C_3-C_6-C_3	Lignans, neolignans
18	C_6-C_6-C_6	Terphenylquinones
19	C_6-C_7-C_6	Diarylheptanoids, curcuminoids
28	$(C_6$-C_2-$C_6)_2$	Bianthrones
30	$(C_6$-C_3-$C_6)_2$	Biflavonoids
n	$(C_6$-$C_3)_n$	Lignin
n	$(C_6$-C_3-$C_6)_n$	Condensed tannins, flavolans
n	$(C_6$-C_1-C_6-$C_1)_n$, $(C_6$-C_1-C_1-$C_6)_n$, $(C_1$-C_6-C_6-$C_1)_n$	Hydrolysable tannins (oligomers)



8-119, phenol,methoxy substituted phenols and their alk(en)yl derivatives

and clove essential oil contains as a key aroma compound eugenol, also known as 2-methoxy-4-(prop-2-en-1-yl)phenol (**8-119**). Eugenol and chavicol (along with myrcene) are major components of the essential oil of allspice (pimento).

3-Alkatrienyl, 3-alkadienyl, 3-alkenyl and 3-alkyl phenols, collectively known as cardanol, occur in the cashew nutshell liquid at a level of 10% (see Section 10.2.3.4). The main components of this mixture are (8*Z*,11*Z*)-3-(pentadeca-8,11,14-trien-1-yl)phenol, (8*Z*,11*Z*)-3-(pentadeca-8,11-dien-1-yl)phenol, (8*Z*)-3-(pentadeca-8-en-1-yl)phenol and 3-pentadecyl phenol. Derivatives of cardanol find applications as polymeric resins, dyestuffs, plasticisers, surface-active agents and pesticides (see Section 8.2.6.1.6). Phenols resulting from pyrolysis of lignin or phenols occurring in liquid smoke (used for smoking foods) are typically found in smoked foods. Pyrolysis of softwood gives rise mainly to guaiacols (2-methoxyphenols, **8-119**), while pyrolysis of hardwood yields a mixture of guaiacols and syringols (2,6-dimethoxyphenols, **8-119**). They not only affect the flavour of smoked products, but stabilise their colour and act as preservatives. The content of phenols in the smoked meat, depending on the smoking process, is around 40 mg/kg. Phenolic substances in alcoholic beverages (such as whisky) are extracted from lignin from oak barrels by ethanol.

Phenolic compounds produced by degradation of phenolic acids or lignin by the activity of microorganisms occur as byproducts of lactic and alcoholic fermentations. Instances of these products are ferulyl alcohol (coniferyl alcohol) and other related alcohols. For example, phenol occurs in sour cream and butter (in butter in an amount of 9–16 μg/kg), and other phenols are present in lower concentrations, especially *m*-cresol (3-methylphenol), *p*-cresol (4-methylphenol) and guaiacol. The content of phenols in alcoholic beverages is highly variable. Concentrations of phenols are relatively low, but they also have low odour detection thresholds (usually within the range of 0.1 to 0.3 mg/l). Phenols only rarely carry an unusual after-taste or off-taste. Higher amounts of phenols are found in various speciality beers (such as beers made from malt directly dried by flue gas), sherry type wines (Table 8.27) and in spirits which are aged in oak barrels. The main phenols in whisky are eugenol, vanillin, guaiacol, phenol, cresols and some other compounds.

Roasting of coffee, nuts or almonds, drying malt, bread baking, production of vegetable oils and other thermal processes generate sensory active volatile phenols that mainly arise from phenolic acids. As an example, the mechanism of phenols formation from ferulic acid is shown in Figure 8.57. At temperatures around 200 °C the main products formed are fragments resulting from the gradual degradation of the three-carbon side-chain at position C-4, its oxidation and partly by recombination of radicals. The composition of these phenols is shown in Table 8.28. The majority of the pyrolytic fragments are produced in the temperature range 360–410 °C. This includes again the cleavage of C–C bonds in the side chain (as at lower temperatures, but to a greater extent than at 200 °C), but the methoxyl group also splits off as a methoxyl radical. The results are three basic structures that represent, in addition to compounds derived from guaiacol, phenol and catechol derivatives. The main guaiacols are 4-ethylguaiacol and 4-vinylguaiacol. The main phenols are 2-methyl-4-vinylphenol, 2-methyl-4-ethylphenol, 4-vinylphenol, 4-ethylphenol, 2,4-dimethylphenol and 4-methyl-2-methylphenol. The main catechols are 4-vinyl-, 4-ethyl, 4-methylpyrocatechols and pyrocatechol.

Recombinations and disproportionations of radicals give rise to other radicals and other products. Some of these reactions are illustrated by the following equations:

$$2CH_3^\bullet \rightarrow CH_3{-}CH_3$$
$$CH_3{-}CH_3 \rightarrow CH_3{-}CH_2^\bullet + H^\bullet$$
$$CH_3{-}CH_2^\bullet \rightarrow CH_2 = CH_2 + H^\bullet$$
$$H_3C^\bullet + H^\bullet \rightarrow CH_4$$
$$H_3CO^\bullet + H^\cdot \rightarrow CH_3OH$$

Analogous products are also formed from cinnamic acid and its derivatives, such as 4-coumaric, caffeic and syringic acids.

Table 8.27 Main phenols contents in selected alcoholic beverages (μg/l).

Compound	Beer	Sherry	Whisky
Phenol	Traces	Traces–100	Traces–12
o-Cresol (2-methylphenol)	Traces	Traces	0–75
m-Cresol (3-methylphenol)	–	10 000	0–35
p-Cresol (4-methylphenol)	–	10 000	9–50
2-Ethylphenol	–	50 000	2
4-Ethylphenol	90	350 000	35–39
2-Methoxyphenol (guaiacol)	10–50	–	0–12
2-Methoxy-4-methylphenol (4-methylguaiacol)	Traces–90	Traces	Traces–5
4-Ethyl-2-methoxyphenol (4-ethylguaiacol)	0–300	80 000	2–36
4-Vinylphenol	5–170	20	–
2-Methoxy-4-vinylphenol (4-vinylguaiacol)	7–100	50	–
4-Allyl-2-methoxyphenol (eugenol)	80	10 000	11–195
4-Ethyl-2,6-dimethoxyphenol	30–1200	40 000	–

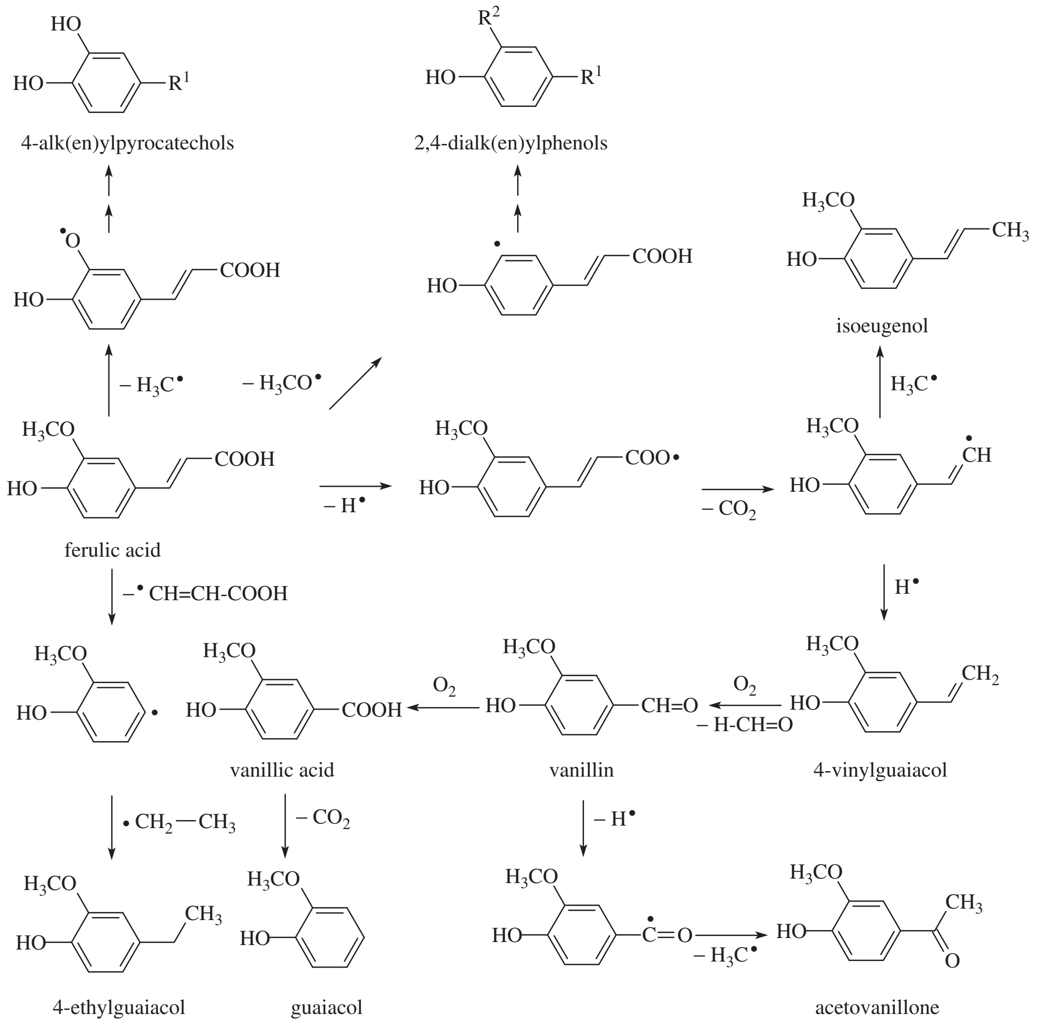


Figure 8.57 Ferulic acid pyrolysis.

Table 8.28 Pyrolysis products of ferulic acid in air at 200 °C.

Product	Composition (%)	Product	Composition (%)
4-Vinylguaiacol	79.9	4-Ethylguaiacol	5.5
Vanillin	6.4	Acetovanillon	2.6
Guaiacol	3.1	Isoeugenol	2.5

For example, the main phenol with antioxidative and antimutagenic activities that occurs in crude canola oil is 2,6-dimethoxy-4-vinylphenol known as canolol, which is produced from syringic acids. The major products of caffeic acid pyrolysis at 225 °C are pyrocatechol, 4-vinylpyrocatechol and 4-ethylpyrocatechol (see Section 8.3.4). A more complex mixture of products arises by pyrolysis of lignin. In the smoke condensates used in the meat industry, more than 150 different phenols, and dozens of aromatic alcohols, phenolic acids and hydroxylated heterocyclic compounds have been identified.

8.2.8.2 Properties and reactions

At higher concentrations, simple phenols are toxic substances that block oxidative phosphorylation. They also exhibit nephrotoxic effects and can act as co-carcinogens. Some alkyl and alkenyl derivatives of phenol, catechol and resorcinol may cause allergic dermatitis.

The most important reaction of natural phenols in foods is oxidation. In many plant foods the enzyme-catalysed oxidation of monophenols to *o*-diphenols (1,2-dihydroxybenzenes) and oxidation of the formed *o*-diphenols to *o*-quinones proceed. These and subsequent reactions belong to the set of enzymatic browning reactions described in Section 9.12.

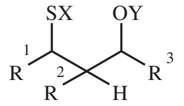
8.2.9 Sulfur compounds

8.2.9.1 Classification structure, terminology and occurrence

Sulfur compounds are an important group of aroma components of foods that determine or significantly affect the desired flavour. However, they may also cause various undesirable off-flavours. Sulfur compounds are often the primary aromatic substances of vegetable foodstuffs (e.g. diallyl disulfide is the characteristic substance of the garlic-like aroma, allyl isothiocyanate that gives the typical aroma of horseradish and 2-isobutylthiazole is a major characteristic component of fresh tomato aroma). Sulfur compounds are formed either from the precursor by enzymatic reactions in damaged plant tissues as primary aromatic substances, or during the heat treatment of foods of plant and animal origin by non-enzymatic reactions as secondarily produced aromatic substances. Sensory active sulfur compounds occur, for example, as important components of the aroma of meat and coffee, but also of many other foodstuffs. A number of sulfur compounds have beneficial effects on human health, especially antimicrobial, antioxidant and anticarcinogenic effects.

Among the most important volatile sulfur compounds are hydrogen sulfide (sulfan), various thiols, sulfides, isothiocyanates and heterocyclic sulfur compounds. Precursors of volatile sulfur compounds are usually non-volatile, sensory indifferent sulfur compounds, especially sulfur-containing amino acids cysteine, cystine, methionine and their derivatives, such as *S*-alk(en)yl cysteine sulfoxides, glucosinolates, thiamine and other compounds. An important sulfur compound is also sulfur dioxide, which is used as a preservative and an inhibitor of enzymatic browning reactions or the Maillard reaction. It also occurs in a small amount as a metabolite in fermentation processes. Sulfur compounds may be accompanied by their selenium analogues at very low concentrations.

Very intense-smelling sulfur compounds with low thresholds of perception often act as key aromatic compounds. They often have very similar structures and contain an important odorophore (a group responsible for the odour character), which is sulfur and oxygen in positions 1 and 3 of the molecule (**8-120**). This structure occurs in mercaptans, thioacetals, mercapto ethers, mercapto ketones, alkylthio esters and other compounds listed in the following sections.



8-120, structure of *S*-compounds with an intense odour

8.2.9.1.1 Sulfane

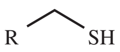
Sulfane (hydrogen sulfide) is present in all foods containing proteins (bound cysteine or cystine) during prolonged storage or heat treatment through the reaction known as desulfuration, or as a byproduct of the Strecker degradation of sulfur-containing amino acids. Sulfane is also formed by degradation of isothiocyanates via carbonyl sulfide and by microbial reduction of sulfates and sulfites. It also occurs in some mineral waters. The human body produces sulfane in trace amounts and uses it as a signalling molecule. Sulfane has a very low odour threshold (below 1 μg/l in air). The odour increases as sulfane becomes more concentrated, and its smell then resembles rotten eggs up to a concentration of about 30 μg/l. Higher concentrations of sulfane in foods that are rich in protein are associated with putrefactive processes. Sulfane is a very reactive compound that produces a number of volatile odour-active products, especially with carbonyl compounds.

8.2.9.1.2 Thiols

Compounds containing a carbon-bonded mercapto group (sulfhydryl group, –SH), called mercaptans or thiols, are the sulfur analogues of hydroxy compounds. Altogether, lower aliphatic thiols are compounds exhibiting an intense, often penetrating,

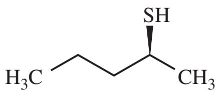
unpleasant and repulsive smell, but at low concentrations they are often important components of the aroma of many foods.

The basic member of the homologous series of thiols is methanethiol (methyl mercaptan, **8-121**), which is produced during the thermal processing of foods, mainly by Strecker degradation of methionine via the unstable primary reaction product methional. Propane-1-thiol (**8-121**) is an important component of leeks and onion flavours, while prop-2-en-1-thiol (allyl mercaptan, **8-121**) is generated during the processing of garlic by dismutation of diallyl disulfide, which is formed from the amino acid alliin via disulfide allicin. At lower concentrations it shows a typical garlic and meat-like aroma. It is also the main active component of 'garlic breath' after eating garlic.

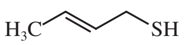


8-121, thiols
methanethiol, R = H
propane -1-thiol, R = CH_2CH_3
prop-2-ene -1-thiol, R = $CH{=}CH_2$
3-methylbutane-1-thiol, R = $CH_2CH(CH_3)_2$
3-methylbut-2-ene-1-thiol, R = $CH{=}C(CH_3)_2$

Foods contain many other aliphatic thiols. The tropical aroma of the guava fruit (*Psidium guava*, Myrtaceae) is credited to (*S*)-pentane-2-thiol (**8-122**), while its higher homologue (*S*)-heptane-2-thiol is a flavour compound found in bell peppers. 3-Methylbut-2-ene-1-thiol (1-mercapto-3-methylbut-2-ene, **8-121**), which arises from prenyl alcohol and hydrogen sulfide, is an important component of roasted coffee aroma. Prenyl alcohol (3-methylbut-2-ene-1-ol) occurs in green coffee at levels of about 0.5 mg/kg. However, the same thiol, 3-methylbut-2-ene-1-thiol (also known as skunky thiol), is a carrier of an off-flavour in beer known as light-struck flavour that appears on exposure of the beer to light (see Section 8.2.12.8.1). Its precursor is the cause of bitter beer taste, iso-α-acids (isohumulones), derived from hops. 3-Methylbut-2-ene-1-thiol results from exposure of beer to UV light and also in a photosensitised reaction (on exposure to sunlight or visible light), where, in the first instance, riboflavin acts as a photosensitiser (see Section 8.3.4.1.4). 3-Methylbut-2-ene-1-thiol in well-stored beer occurs a level of 1–5 ng/l, but in the light its content increases up to 0.01–1.05 μg/l. At present modified (hydrogenated) iso-α-acids are produced, enabling cold-hopping and providing increased resistance against the formation of this compound after irradiation of the beer. For example, the major volatile components of the anal sac secretion from North American skunks are similar mercaptans, namely (*E*)-but-2-ene-1-thiol (**8-123**) and 3-methylbutane-1-thiol (**8-121**) that comprise 18–66% of the secretion.

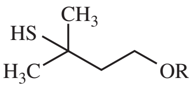


8-122, (*S*)-pentane-2-thiol

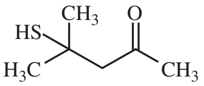


8-123, (*E*)-but-2-ene-1-thiol

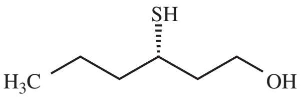
Not all thiols have unpleasant odours. A component of raw and heat-treated onion with a very intense aroma is 3-mercapto-2-methylpentan-1-ol (2-methyl-1-hydroxypentan-3-thiol, see Section 8.2.12.6.4). The characteristic sulfur note of blackcurrants is caused by the presence of 4-methoxy-2-methylbutan-2-thiol (4-methoxy-2-methyl-2-mercaptobutane), called blackcurrant mercaptan (**8-124**), which occurs in aromatic juices at concentrations of 0.2 μg/l and higher. It seems that it can be used to express a fruity aroma to tropical fruit juices, coffee and other products. The same compound is also found in virgin olive oils at levels of about 2 μg/l. Its odour threshold is 0.045 μg/kg in oil. Another component resembling blackcurrant aroma is 4-mercapto-4-methylpentan-2-one, the so-called cat ketone (**8-125**). The same compound also occurs in small amounts in grapefruits, Japanese green tea, some hop cultivars, basil and certain aromatic wines (such as Sauvignon Blanc), which contributes to their musky aroma. In passion fruit (*Passiflora edulis*, Passifloraceae) contributions to the aroma come from 3-mercaptohexan-1-ol along with a number of other sulfur compounds, such as 3-mercaptohexyl acetate, 3-mercapto-3-methylbutan-1-ol and 3-mercapto-3-methylbutyl acetate. Both enantiomers of 3-mercaptohexan-1-ol also occur in some white wines. The two enantiomers differ in their odour, the (*S*)-enantiomer (**8-126**) smells more of passion fruit, while the (*R*)-enantiomer is fruitier and reminiscent of grapefruit. The odour threshold is 0.08 ng/l in air. Alicyclic thiols are also significant aromatic compounds. The characteristic flavour component of grapefruit is monoterpene *p*-mentha-1-ene-8-thiol. Another related thiol, (1*S*,4*R*)-*p*-methane-8-thiol-2-one (see Section 8.2.13.1.1) is a key component of the scent of blackcurrant.



8-124, 3-mercapto-3-methylbutan-1-ol, R = H
4-methoxy-2-methylbutane-2-thiol, R = CH_3



8-125, 4-mercapto-4-methylpentan-2-one



8-126, (+)-(*S*)-3-mercaptohexan-1-ol

Volatile thiols are present in fruits and vegetables in combined forms, as *S*-cysteine and *S*-glutathione conjugates, and are released from these precursors by the action of L-cysteine-*S*-conjugate thiol-lyase (deaminating) during fermentation (Figure 8.58). For example, the precursors of 3-mercaptohexan-1-ol in passion fruit and wines are the individual diastereomers of 3-*S*-(hexan-1-ol)-L-cysteine and in wines, correspondingly, diastereomers of 3-*S*-glutathionylhexan-1-ol. The precursor of 4-mercapto-4-methylpentan-2-one in wines is 4-*S*-glutathionyl-4-methylpentan-2-one.

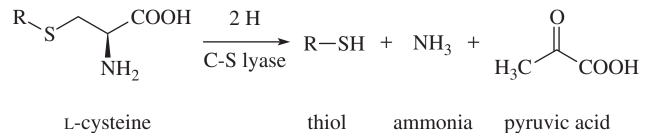
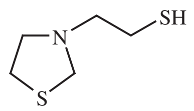


Figure 8.58 Formation of thiols by degradation of cysteine conjugates.

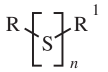
Thiols arising in the Maillard reaction, mostly derived from heterocyclic compounds, such as furan, thiophene, thiazole and other heterocycles, are important flavour-active components of meat, coffee and many other foods. For example, 2-furanmethanethiol (furan-2-ylmethanethiol, furfuryl mercaptan) has an odour resembling roasted coffee, *N*-(2-mercaptoethyl)-1,3-thiazolidine (**8-127**), which is formed in the reaction of fructose with cysteamine, and has a very strong odour resembling popcorn. Its odour threshold is 0.005 ng/l in air.



8-127, 3-(2-mercaptoethyl)-1,3-thiazolidine

8.2.9.1.3 Sulfides and oligosulfides

In foods, a range of aliphatic sulfides, disulfides, trisulfides (**8-128**) and higher oligosulfides, but also some cyclic sulfides, are important compounds. Sulfides and oligosulfides have an unpleasant odour at higher concentrations, but in low concentrations they contribute to the characteristic full flavour of many foods, such as onion, garlic, cheese, meat, vegetables (especially *Brassica* vegetables) and chocolate, and are used in various flavour compositions.



8-128, sulfides

sulfides, $n = 1$
disulfides, $n = 2$
trisulfides, $n = 3$
dimethyl sulfide, $n = 1$, $R = R^1 = CH_3$
dimethyl disulfide, $n = 2$, $R = R^1 = CH_3$
dimethyl trisulfide, $n = 3$, $R = R^1 = CH_3$
di(prop-2-en-1-yl)trisulfide, $n = 3$, $R = R^1 = CH{=}CH{-}CH_3$

Dialkyl sulfides are sulfur analogues of ethers. They are formed in foods (along with dialkyl trisulfides) mainly by disproportionation of disulfides. The homolytic S–R bond cleavage of disulfides proceeds by photolysis or heating:

$$R{-}S{-}S{-}R \rightarrow R{-}S{-}S^{\bullet} + R^{\bullet}$$
$$R{-}S{-}S{-}R + R^{\bullet} \rightarrow R{-}S{-}R + R{-}S^{\bullet -}$$
$$R{-}S{-}S{-}R + R{-}S^{\bullet} \rightarrow R{-}S{-}S{-}S{-}R + R^{\bullet}$$

The basic member of the homologous series of dialkyl sulfides is dimethyl sulfide, presenting an odor threshold of 0.12 μg/l in water, which has a characteristic sulfurous, cabbage-like smell at low concentrations, similar to that of dimethyl disulfide, which also has garlic-like notes. Dimethyl trisulfide odour is described as sulfurous, alliaceous, cooked, savoury, meaty, eggy and vegetative, with a fresh, green and onion top note. Dimethyl sulfide (**8-128**, $R{=}R^1{=}CH_3$, $n{=}2$) arises by disproportionation of dimethyl disulfide (e.g. in garlic and cruciferous vegetables). In many vegetables (such as cooked cabbage, celery and some other vegetables), dimethyl sulfide forms by decomposition of *S*-methylmethionine during food storage and/or thermal treatment. *S*-Methylmethionine, known as vitamin U (Figure 8.59), is a natural component of *Brassica* and other vegetables (see Section 5.15). In cruciferous vegetables, dimethyl disulfide and trisulfide result from degradation of *S*-methylcysteine sulfoxide. Dimethyl sulfide is an important aromatic component of tea, coffee, cocoa and other foods; however, in certain foods it causes an unpleasant off-flavour (as in beer and milk). For example, sulfides contribute to an off-flavour of aged beer resembling cooked vegetables, especially creamed maize, cabbage and tomato, and shellfish/oyster-like in high concentrations. The main undesirable compounds responsible for this off-flavour include dimethyl disulfide (first perceived at concentration of about 30 μg/l), diethyl sulfide, diisopropyl sulfide, dimethyl sulfide and other compounds at considerably lower concentrations. Dimethyl disulfide and trisulfide (**8-128**) are, along with degradation products of glucosinolates, important components of cruciferous vegetables aroma. Dimethyl trisulfide contributes significantly to the flavour of chicken, cabbage and garlic.

Ethyl methyl disulfide, along with other sulfur compounds, is a flavour-active component of the highly prized fruit durian (*Durio zibethinus*, Bombacaceae), native to Indonesia and Malaysia, whose odour is reminiscent of rancid meat. Allyl-, propyl-, methyl- and

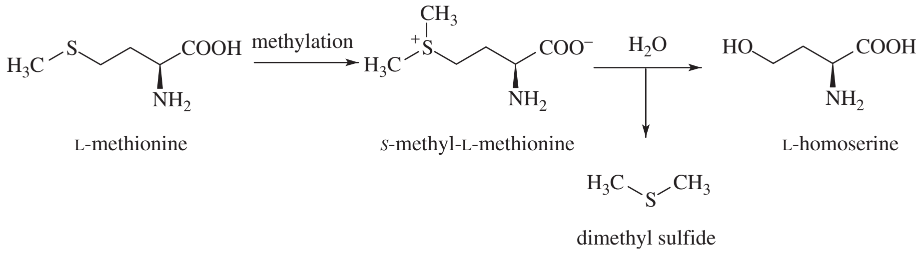
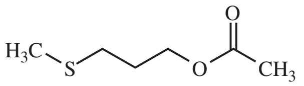


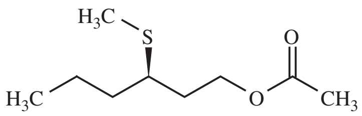
Figure 8.59 Formation of dimethyl sulfide from *S*-methyl methionine.

prop-1-en-1-yl sulfides (mono-, di-, tri- and tetrasulfides) are some of the most important sensory active components of bulb vegetables, such as garlic, chives, leeks, onions, shallots and other varieties of onions, where they arise by enzymatic (and also by thermal) decomposition of *S*-alk(en)ylcysteine sulfoxides, mainly via the corresponding thiosulfinates.

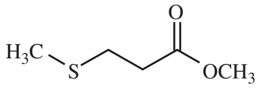
An important sulfide is methional (**8-37**). Methional in beer and wine is formed by the activity of microorganisms. It is partly reduced to the corresponding alcohol methionol (**8-13**) and reaction with acetyl-CoA yields 3-methylthiopropyl acetate (**8-129**), which is an important component of various fermented foods. Another ester of acetic acid 3-(methylthio)hexyl acetate is a component that posseses attractive tropical fruity notes on dilution. The less odoriferous (−)-(*R*)-enantiomer (**8-130**) is reminiscent of passion fruit, while the (+)-(*S*)-form has a more herbaceous odour. The odour thresholds of these thiols in air are 0.10 ng/l and 0.03 ng/l, respectively. Both isomers have been found in passion fruit (*Passiflora edulis*, Passifloraceae), guava (*Psidium guajava*, Myrtaceae) and aromatic white wines. Methyl-3-(methylthio)propionate, or pineapple mercaptan (**8-131**), has a flavour reminiscent of pineapple. *S*-Methylthiohexanoate (**8-132**) is a component of the durian fruit smell. Condensation of methional with ethanol yields (*Z*)-2-(methylthio)methylbut-2-enal also known as 2-ethylidenemethional (**8-133**), which is an important component of potato chips aroma. It also occurs in



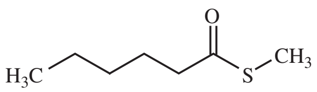
8-129, 3-(methylthio)propyl acetate



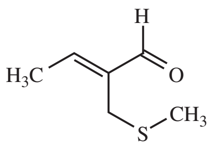
8-130, (*R*)-3-(methylthio)hexyl acetate



8-131, methyl 3-(methylthio)propionate

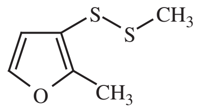


8-132, *S*-(methylthio)hexanoate



8-133, (*Z*)-2-(methylthiomethyl)but-2-enal

coffee, cocoa and other foods. Methyl (2-methyl-3-furyl) disulfide (**8-134**) is a component of meat volatiles, and also of chocolate aroma. Many other sulfides are, similarly, important components of foods.



8-134, methyl(2-methyl-3-furyl)disulfide

Cyclic disulfides, for example, dithiines (see Section 8.2.12.6.4), are components of garlic aroma. The mushroom shiitake (*Lentinula edodes*), native to East Asia, with a sulfurous aroma, contains 4,4,7,7-tetramethyl-1,2,3,5,6-pentathiepan known as lenthionine (see Section 8.2.12.9) as a characteristic component.

8.2.9.1.4 Thiosulfinates

Thiosulfinates of a general structure R–SO–S–R, also known as alkanethiosulfinic acid esters, are the basic group of oxidised disulfides. In foods, thiosulfinates may result from oxidation of disulfides, for example, by fatty acid hydroperoxides, as in the case of cystine (see Section 2.5.1.1.1). Precursors of a number of acyclic and cyclic thiosulfinates and other volatile sulfur compounds in plants are *S*-alk(en)ylcysteine sulfoxides (see Section 2.2.1.2.2) that typically occur in garlic, onion and other plants of the *Amarillidaceae* family. These amino acids are decomposed enzymatically to alk(en)ylsulfenic acids under the catalysis of C–S lyases (cysteine sulfoxide lyase known as alliinase that is found in garlic and other Alliums). Alk(en)ylsulfenic acids then undergo very facile self-condensation to dialk(en)ylthiosulfinates. Dimethylthiosulfinate is produced in Amarillidaceae family vegetables and also in plants of the Brassicaceae family vegetables from *S*-methylcysteine sulfoxide via methylsulfenic acid. A well known thiosulfinate is diallylthiosulfinate, which is di(prop-2-en-1-yl)thiosulfinate (called allicin), which is formed via (prop-2-en-1-yl)sulfenic acid from *S*-allylcysteine sulfoxide (alliin) found mainly in garlic. Allicin is a chiral compound, but occurs naturally only as a racemate. Analogously, but to a lesser extent, other thiosulfinates are also found in garlic, for example dimethylthiosulfinate, allylmethylthiosulfinate and methylallylthiosulfinate. Another important thiosulfinate is di(prop-1-en-1-yl)thiosulfinate, which is found mainly in onion via (*E*)-(prop-1-en-1yl)sulfenic acid from (*E*)-*S*-(prop-1-en-1-yl)cysteine sulfoxide (isoalliin). The general mechanism for the formation of dialk(en)ylthiosulfinates is given in Figure 8.60.

Dialk(en)ylthiosulfinates are relatively labile compounds. Their degradation is rapid even under room temperature and is influenced by various factors. For example, the half-life of allicin in water is 30–40 days at 23 °C. In non-polar media, such as organic solvents and vegetable oils, allicin is transformed into the corresponding main products dithiines (about 70%), diallyl disulfide and diallyl oligosulfides (about 18%), 4,5,9-trithiadodeca-1,6,11-triene 9-oxides known as ajoenes (ajo is the Spanish word for garlic) and certain other products (Figure 8.61). Vinyldithiines occur as regioisomers, 2-vinyl-4*H*-1,3-dithiine (the main product)

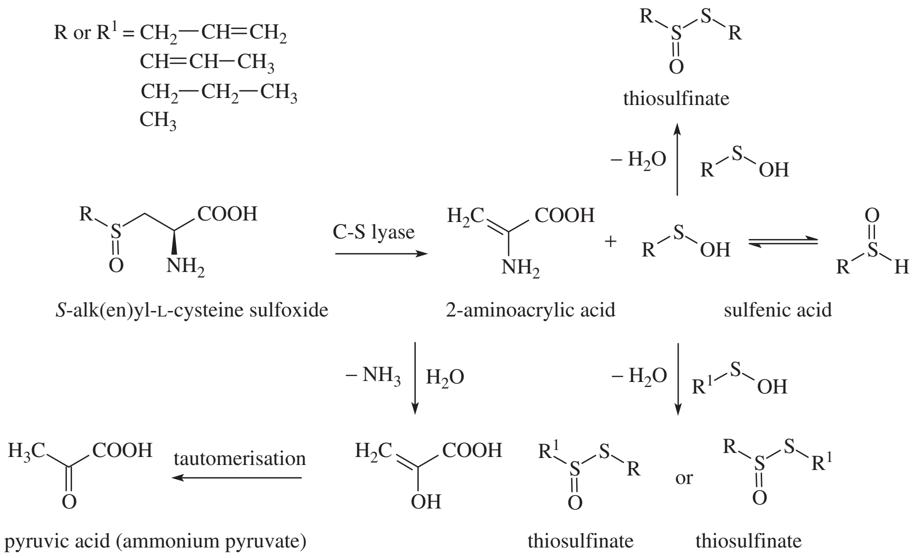


Figure 8.60 Formation of dialk(en)ylthiosulfinates by enzymatic decomposition of *S*-alk(en)ylcysteine sulfoxides.

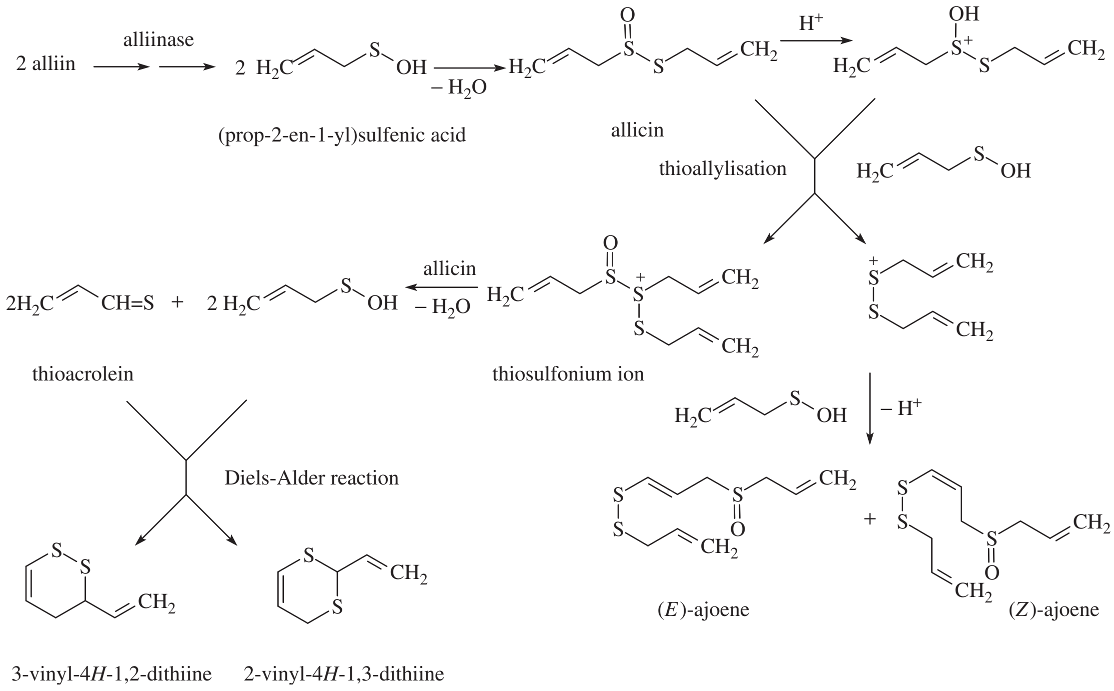


Figure 8.61 Transformation of allicin to dithiines and ajoenes in non-polar media (see Figure 8.57). Block, Dane, Thomas and Cody, 2010, fig 1. Reproduced by permission of the American Chemical Society.

and 3-vinyl-4*H*-1,2-dithiine, with (*E*)-ajoene usually present in double the amounts of (*Z*)-ajoene.

The main products of alliin transformation in polar media are sulfides. At room temperature or on heating, allicin is converted by water into diallyl disulfide, diallyl trisulfide and diallyl polysulfides, which are the principal components of garlic essential oil and aged garlic (Figure 8.62). Other reaction products are allyl alcohol, sulfur dioxide and propene. Disproportionation of thiosulfinates in aqueous (polar) solutions also leads to disulfides and thiosulfonates of general formula R–SO_2–S–R. For example, disproportionation of allicin yields diallylthiosulfonate (pseudoallicin) and diallyl disulfide, which subsequently give diallyl sulfide and diallyl trisulfide. In addition to alliin, garlic contains lesser amounts of methiin and isoalliin, which decompose by alliinase to the corresponding alk(en)ylsulfenic acids. These acids can self-condense to the corresponding dialk(en)ylthiosulfinates or react with allicin to yield mixed alk(en)ylthiosulfinates.

The (*E*)-(prop-1-en-1-yl)sulfenic acid derived from the related amino acid occurring in onion and known as isoalliin behaves differently. It isomerises on catalysis with enzyme LF-lyase

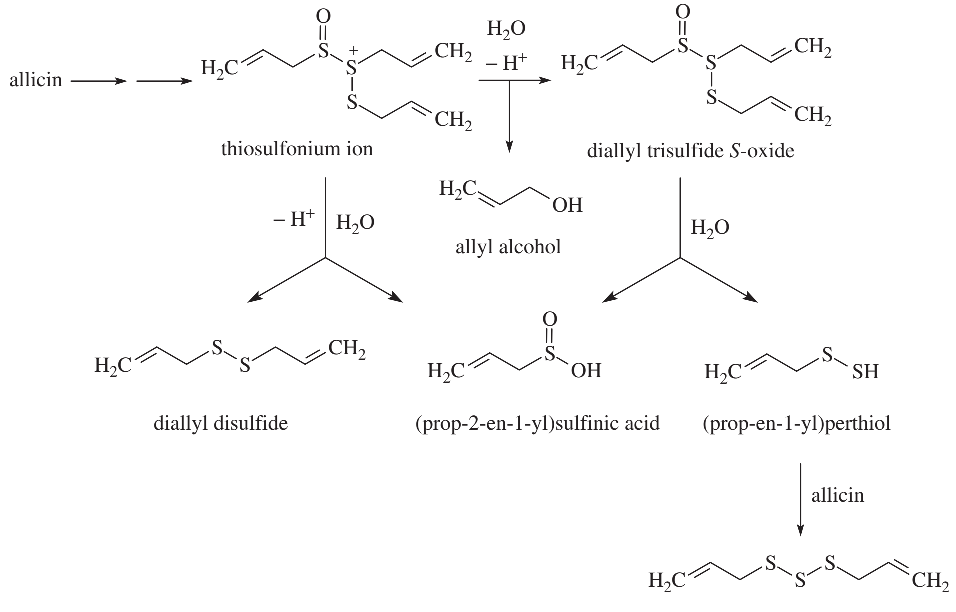
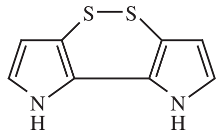


Figure 8.62 Transformation of allicin to oligosulfides and allyl alcohol in polar media (see Figure 8.58). Block, Dane, Thomas and Cody, 2010, fig 1. Reproduced by permission of the American Chemical Society.

(Lachrymatory Factor-lyase), absent in garlic, to (*Z*)-thiopropanal *S*-oxide (thiopropanal sulfoxide), which is a lachrymatory substance that causes tears when cutting onions (Figure 8.63). Leek and chives contain the same sulfur-containing amino acids as onion; only the propiin content is somewhat higher at the expense of isoalliin, which is the major cause of much lower lachrymatory properties of these vegetables. (*E*)-(Prop-1-en-1-yl)sulfenic acid or (*Z*)-thiopropanal sulfoxide yield (*Z*,*Z*)-2,3-dimethyl-1,4-butanedithial 1,4-dioxide, which is also known as bis-sulfine. Dehydration of two molecules of (*E*)-(prop-1-en-1-yl)sulfenic acid produce (prop-1-en-1-yl)thiosulfinate, which is the precursor of 2,3-dimethyl-5,6-dithiabicyclo[2.2.1]hexane 5-oxides, known as zwiebelanes (Zwiebel is the German word for onion). The main product is (*E*)-zwiebelane, which is a chiral isomer. Spontaneous cyclisation of isoalliin in raw and cooked onion gives (1*S*,3*R*,5*S*)-3-carboxy-5-methyl-1,4-thiazan-*S*-oxide, also known as cycloalliin (see Section 2.2.1.2.7).

Technological processing of garlic, onions and leeks (e.g. cutting, preservation in salt or drying) often leads to the formation of undesirable intensely coloured compounds that are pink to deep red with onions and leeks, and dark green or green–blue with garlic. These pigments are not stable and are slowly converted into yellow–brown or brown pigments within a few days of storage. It was found that the pinking of onions and leeks and the greening of garlic are chemically very similar reactions consisting of enzymatic and non-enzymatic processes. The primary precursor is the sulfur amino acid isoalliin (see Section 2.2.1.2.2). In disrupted tissues, isoalliin is, along with other *S*-substituted cysteine derivatives, degraded by the enzyme alliinase to form (prop-1-en-1-yl)thiosulfinate, $H_3C–CH=CH–S(=O)–S–CH=CH–CH_3$, or mixed thiosulfinates, $H_3C–CH=CH–S–S(=O)–R$ ($R=CH_2CH=CH_2$ or CH_3). These compounds then react with free amino acids to form pigment precursors, *N*-substituted 3,4-dimethylpyrroles. Molecules containing a 3,4-dimethyl pyrrole ring are then cross-linked by an allyl group of allicin (or with thioacrolein formed from allicin, Figure 8.61) to form conjugated reddish-purple pigments (Figure 8.63). *N*-Substituted 3,4-dimethylpyrroles derived from alanine and valine have been identified in onion. A colour shift towards blue to green can be expected as the cross-linking reaction continues to form, for example, tri- or tetrapyrrole compounds.

Most of the species of the subgenus *Melanocrommyum* of the genus *Allium* are characterised by a deep orange to red ichor (fluid discharge) occurring after damage of the cells. This red pigment, 3,3′-dithio-2,2′-dipyrrole (**8-135**), forms spontaneously from (R_C,S_S)-*S*-(pyrrol-3-yl)cysteine sulfoxide (see Section 2.2.1.2.2) via 2-lactyl-3′-pyrrolyl sulfoxide.



8-135, 3,3′-dithio-2,2′-dipyrrole

8.2.9.1.5 Isothiocyanates

Isothiocyanates (formerly known as mustard oils, **8-136**) are compounds with a cumulated system of double bonds belonging to the heterocumulenes, compounds that are formally derived from the hydrocarbon allene ($H_2C=C=CH_2$). In small quantities, isothiocyanates are often accompanied by isomeric thiocyanates. Related cyanates and isocyanates do not occur in foods.

Precursors of isothiocyanates in food are the sulfur-containing glycosides known as glucosinolates that are found in the

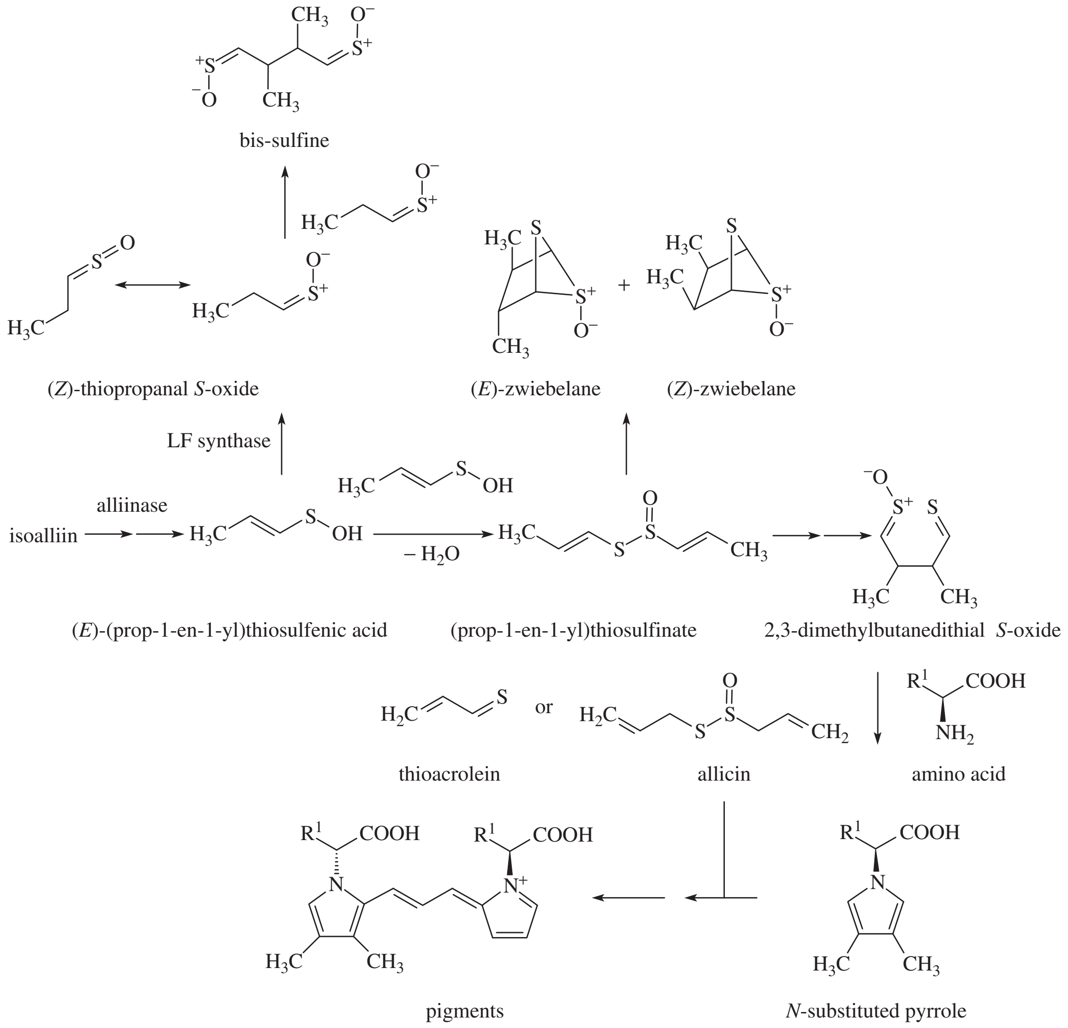
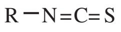


Figure 8.63 Transformation of isoallicin to (*Z*)-thiopropanal *S*-oxide, zwiebelanes and coloured products. Block, Dane, Thomas and Cody, 2010, fig 1. Reproduced by permission of the American Chemical Society.



8-136, isothiocyanates

cruciferous plant family (Brassicaceae) in particular. When the plant is damaged, for example by cutting or masticating, the released enzyme β-glucosidase is released to catalyse the glucosinolate degradation to isothiocyanates and a number of other substances. Isothiocyanates are substance with a very intense and sharp odour and taste. One of the major representatives of this group of substances is allyl isothiocyanate (prop-2-en-1-yl isothiocyanate, **8-136**, R=CH_2–CH=CH_2), which is an active component of hot horseradish, pastes made from brown and black mustard seeds and a number of *Brassica* vegetables.

8.2.9.2 Properties and reactions

Sulfur compounds are reactive and enter into reactions with other food components. Most sulfur compounds are substances with characteristic intense flavours that contribute to the odour and taste of many foods.

8.2.9.2.1 Sulfane, thiols and sulfides

Sulfane and thiols react readily with the carbonyl groups of carbonyl compounds (Figure 8.26) and they often undergo addition reactions to double bonds of unsaturated compounds. Unlike alcohols, thiols are easily oxidised by atmospheric oxygen to disulfides. The reaction mechanism is given in Section 2.5.1.1.1. Like methionine, cysteine and cysteine, simple sulfides and disulfides are also oxidised to sulfoxides, sulfones and other products. Fatty acid hydroperoxides are important oxidation reagents generated in the autoxidation of unsaturated fatty acids. An important reaction of oligosulfides is the interchange reaction that also proceeds in peptides and proteins containing bound cystine:

$$R–S–S–R + R^1–S–S–R^1 \rightarrow 2R–S–S–R^1$$

In veterinary science, it is known that onion and garlic are oxidatively toxic to erythrocytes resulting in haemolytic anaemia

in domestic animals, such as dogs, cats, horses, sheep and cattle. The causative agents have been identified as di(prop-2-en-1-yl) trisulfide (**8-128**), tetrasulfide and pentasulfide, di(prop-2-en-1-yl)thiosulfonate (R–SO_2–S–R, R = prop-2-en-1yl) and other transformation products of isoalliin.

8.2.9.2.2 Thiosulfinates

Aliphatic thiosulfinates formed by enzymatic decomposition of *S*-alk(en)yl cysteine sulfoxides show antimicrobial and antiaggregatory activity with human blood platelets, but are rather unstable. They are mainly oxidised to less active thiosulfonates. Thiosulfinates derived from vegetables such as garlic and onions can freely permeate cell membranes and rapidly react with reduced glutathione (G–SH) to form intracellular mixed-disulfide conjugates of the type G–S–S–R, where R = allyl or propyl. Analogous conjugates with cystine are found in extracellular fluids. Conjugation reactions of thiosulfinates in the presence of free thiols, including those in proteins, also occur in foods. Some conjugates of thiosulfinates with cysteine and degradation products of thiosulfinates have demonstrable biological activities. For example, *S*-allylmercaptocysteine (Cy–S–S–allyl) is believed to be a major bioactive component in aged garlic extract and has antioxidant, antiproliferative, apoptosis-inducing and antimetastatic activities. Another active compound is *S*-allylmercaptoglutathione (G–S–S–allyl). Decomposition products of thiosulfinates similarly have various physiological effects, such as lowering of blood pressure, cholesterol and blood sugar levels, antimutagenic and anticarcinogenic activities.

8.2.9.2.3 Isothiocyanates

Isothiocyanates are not only flavour-active compounds, but also have some antibacterial and fungicidal effects and a certain toxicity, such as mild strumigenic and cytotoxic activities. These properties are found especially in methyl isothiocyanate and allyl isothiocyanate, which are used as pesticides as their insecticidal, herbicidal, fungicidal and nematocidal effects can control soil-borne plant pathogens and parasitic nematodes. Allyl isothiocyanate have found use in the canning industry in modified atmospheres to extend the shelf life of packaged food (e.g. of meat products). The ability of some isothiocyanates of cruciferous vegetables to inhibit chemically induced cancer has also been demonstrated. These isothiocyanates are therefore classified as natural anticarcinogenic agents. In this respect, particular attention has been paid to sulforaphane (4-methylsulfinylbutyl isothiocyanate), which arises from its precursor, glucoraphanin, in broccoli and radishes (see **10-88**).

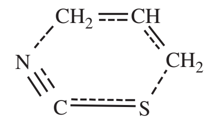
In aqueous solutions, isothiocyanates rearrange into thiocyanates. The rearrangement takes place via various mechanisms, usually by heterolytic or homolytic cleavage of the molecule (Figures 8.64 and 8.65). Allyl isothiocyanate preferably isomerises via the six-membered cyclic allyl thiocyanate intermediate (**8-137**). Isothiocyanates are extremely reactive substances, due to the electrophilic nature of their functional group, and enter into reactions with a number of nucleophilic reagents. Particularly important reactions in foods are the reactions of isothiocyanates with mercapto, amino and hydroxyl groups of amino acids and proteins. Some reactions of isothiocyanates with amino acids also produce coloured products (see Section 2.5.2.4).

$$R{-}N{=}C{=}S \rightleftharpoons R^+ + [{}^-S{=}C{=}N \longleftrightarrow {}^-S{-}C{\equiv}N] \rightleftharpoons R{-}S{-}C{\equiv}N$$

Figure 8.64 Heterolytic cleavage of isothiocyanates.

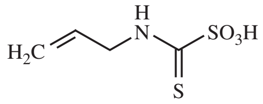
$$R{-}S{-}C{\equiv}N \rightleftharpoons R\bullet + SCN\bullet$$
$$R\bullet + R{-}N{=}C{=}C \rightleftharpoons R\bullet + R{-}S{-}C{\equiv}N$$

Figure 8.65 Homolytic cleavage of isothiocyanates.



8-137, cyclic intermediate involved in the isomerisation of allyl isothiocyanate to allyl thiocyanate (transition state)

Additions of various nucleophilic reagents to the isothiocyanate functional group are shown in Figure 8.66. The nucleophilic reagent can be water (hydroxyl ions), which yields thiocarbamoic acid salts, whereas alcohols give thiocarbamic acid esters, sulfane gives dithiocarbamoic acid salts, thiols give dithiocarbamoic acid esters, ammonia gives *N*-substituted thioureas and amines give *N,N′*-disubstituted thioureas. Reactions are usually complex and depend on pH and other factors. The main reactions of allyl isothiocyanate in aqueous solutions are shown in Figure 8.67. The main product in weakly acidic solutions (pH 4) is carbon disulfide and allylamine. In slightly alkaline solutions (pH 8) allylamine, allyldithiocarbamate, diallylthiourea and carbon disulfide form as the main products. Diallyl disulfide and other products are also produced in small amounts. For example, diallyl disulfide imparts a faint odour resembling garlic to older mustard pastes. In the presence of hydrogen sulfites (HSO_3^- ions), sometimes used as preservatives, allyl isothiocyanate yields allylaminothiocarbonylsulfonic acid (**8-138**).



8-138, allylaminothiocarbonyl sulfonic acid

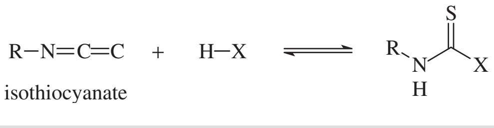


Figure 8.66 Reaction of isothiocyanates with nucleophilic reagents: HX = water (H_2O) – thiocarbamoic acid, alcohol (R^1–OH) – thiocarbamate, sulfan (H_2S) – dithiocarbamoic acid, ammonia (NH_3) – *N*-alkylthiourea, amine (R^1-NH_2) – *N,N*[1]-dialkylthiourea.

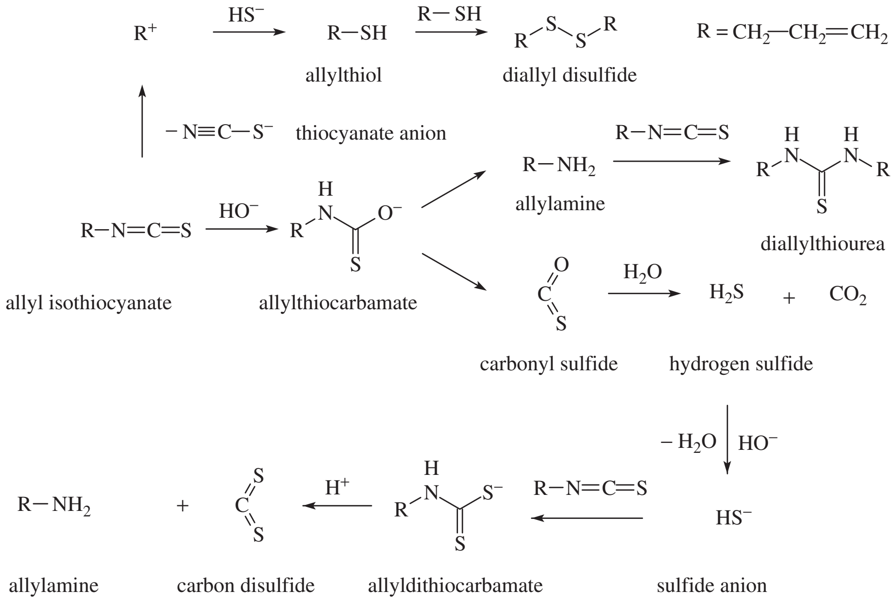


Figure 8.67 Decomposition of allyl isothiocyanate in aqueous solutions.

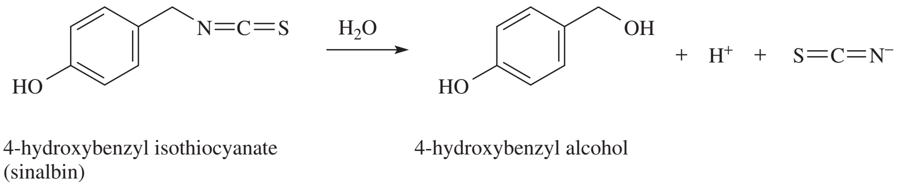


Figure 8.68 Hydrolysis of sinalbin.

Aromatic and heterocyclic isothiocyanates are unstable and easily hydrolyse to the corresponding alcohols. For example, 4-hydroxybenzyl isothiocyanate (the degradation product of sinalbin, which occurs in the seeds of white mustard, *Leucosinapis album*, Brassicaceae) decomposes in normal table mustard to 4-hydroxybenzyl alcohol and thiocyanate ions (Figure 8.68). Isothiocyanates derived from indolyl glucosinolates behave similarly.

8.2.10 Nitrogen compounds

8.2.10.1 Classification, structure, terminology and occurrence

Ammonia, volatile amines imines and amides and, in particular, heterocyclic compounds containing nitrogen, have certain importance as flavour-active substances in non-acidic foods.

8.2.10.1.1 Ammonia

Ammonia (NH_3) is found in foods mainly as a product of free nucleotide deamination, such as deamination of adenosine 5′-monophosphate (AMP) to inosine 5′-monophosphate, and as a product of deamination of the amino acid amides asparagine and glutamine. In acidic foods, ammonia is present almost exclusively in the form of ammonium salts.

8.2.10.1.2 Amines

Amines are structurally derived from ammonia by substitution. The basic amine groups are primary amines (RNH_2), secondary amines (RR^1NH), tertiary amines (RR^1R^2N) and quaternary amines with the general structure $RR^1R^2R^3N^+(OH^-)$. Tertiary amines can form *N*- oxides of the type $RR^1R^2N^+(O^-)$.

Primary amines in foods most often arise as products of enzymatic reactions that include decarboxylation of amino acids catalysed by non-specific decarboxylases or enzymatically catalysed amination or transamination of aldehydes (Figure 8.69).

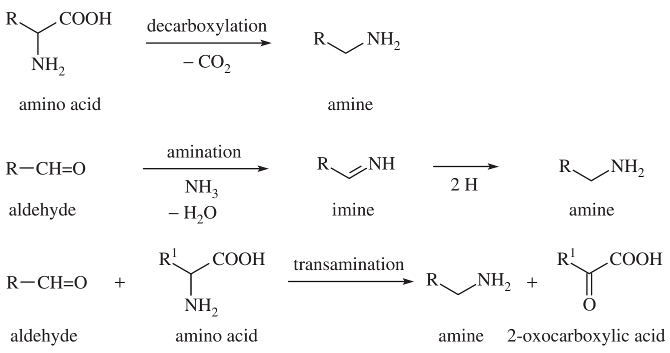
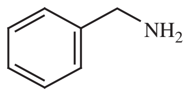


Figure 8.69 Main reactions of amine formation.

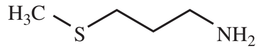
The decarboxylation of amino acids takes place mainly in materials of animal origin, while the formation of aldehydes and amines occurs largely in plant materials. Common products are aliphatic amines. In aromatic compounds, such as benzylamine (**8-139**), the amino group is bound in the side-chain. Amines are present in virtually all foods, and therefore can also be found in fruits, alcoholic beverages and so on, but they act as odour-active substances only in some non-acidic foods of animal origin, especially in cheeses, fish, other aquatic animals and meat. In acidic foods, amines are present in the form of non-volatile salts. In plants and fungi, amines often have a function of insect attractants. An overview of major amine precursors is given in Table 8.29. The common volatile amines contents in selected foods are shown in Table 8.30. The enzymatic decarboxylation also occurs in amino acids other than those listed in Table 8.29. Sulfur amino acids and hydroxyamino acids are decomposed with the formation of non-volatile amines. For example, cysteamine (2-mercaptoethylamine, **8-140**) is produced from cysteine, homocysteamine (3-mercaptopropylamine, **8-133**) from homocysteine, 3-(methylthio)propylamine (**8-141**) from methionine, ethanolamine (2-aminoethanol, **8-142**) from serine and (−)-(*R*)-1-aminopropan-2-ol, (**8-142**, R=CH_3) from threonine. Ethanolamine also results from the hydrolysis of some glycerophospholipidis, for example from (3-*sn*-phosphatidyl)ethanolamine.

Some aliphatic, aromatic and heterocyclic amines and diamines formed by decarboxylation of basic, aromatic and heterocyclic amino acids are biologically active substances called biogenic amines. Subsequent enzymatic transformations of biogenic amines yield other biologically active products that play important roles in living organisms, such as adrenal hormones called catecholamines (see Section 10.3.2.10.1).



8-139, benzylamine

8-140, cysteamine, $n = 0$
homocysteamine, $n = 1$



8-141, 3-(methylthio)propylamine

ethanolamine (*R*)-1-aminopropan-2-ol

8-142, amino alcohols

Decarboxylation of amino acids can also proceed as a non-enzymatic reaction. Analogously to the enzyme catalysed decarboxylations, amines are formed as byproducts of the Strecker degradation of amino acids and by thermal decarboxylation of amino acids, especially of sulfur amino acids, hydroxyamino acids and aromatic amino acids. For example, thermal decarboxylation and subsequent reactions of cysteine and cystine produce ammonia

Table 8.29 Precursors of volatile amines in foods.

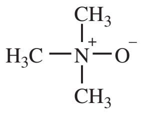
Amine	Precursor		Amine	Precursor	
	Amino acid	Aldehyde		Amino acid	Aldehyde
Methylamine	Glycine	Formaldehyde	Butylamine	Norvaline	Butanal
Ethylamine	Alanine	Acetaldehyde	Pentylamine	Norleucine	Pentanal
Propylamine	2-Aminobutyric	Propanal	Isopentylamine	Leucine	3-Methylbutanal
Isobutylamine	Valine	2-Methylpropanal	Hexylamine	1-Aminoheptanoic	Hexanal
2-Methylbutylamine	Isoleucine	2-Methylbutanal	Benzylamine	Phenylglycine	Benzaldehyde

Table 8.30 Ammonia and volatile amines contents in selected foods.

Compound	Content (mg/kg fresh weight)				Compound	Content (mg/kg fresh weight)			
	Cabbage	Carrot	Cheeses	Beer		Cabbage	Carrot	Cheeses	Beer
Ammonia	15 260	3970	16 440	10–68	Isobutylamine	-	-	0.2	<0.22
Methylamine	16.6	3.8	3–12	0.02–0.32	Pentylamine	0.4	-	1.2	-
Ethylamine	1.3	1	1–4	0.31–2.12	Isopentylamine	0.5	-	<0.2	<0.14
Propylamine	-	-	2–8.7	<0.17	Dimethylamine	2.8	-	-	0.6
Butylamine	-	-	3.7	<0.07	Benzylamine	3.8	2.8	-	-

and ethylamine and ethylamine, pentylamine and crotylamine (but-2-en-1-ylamine) arise from methionine. A number of aromatic and heterocyclic amines that have the amino group attached to the aromatic (heterocyclic) ring may be formed by pyrolysis of amino acids and proteins in the Maillard reaction, especially in the presence of creatinine. Some of these amines (e.g. aminoimidazoazaarenes), described in Section 12.2.1.1, are mutagenic and probably carcinogenic compounds.

Secondary and tertiary amines are formed from precursors other than amino acids. Dimethylamine results from degradation of choline (which is present in some phospholipids), some alkaloids (e.g. in beer it is produced from gramine (see **10-198**) present in germinating barley grains and also in non-enzymatic browning reactions from methylamine and formaldehyde or by decarboxylation of sarcosine. Trimethylamine, together with dimethylamine, methylamine and ammonia, is an odorous compound of fish and other aquatic animals. It is formed by reduction of the sensory indifferent trimethylamine oxide (trimethylaminoxide, **8-143**) in tissues post mortem.

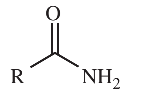


8-143, trimethylamine oxide

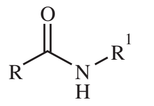
8.2.10.1.3 Amides

Amides derived from carboxylic acids (**8-144**) and the corresponding *N*-substituted (**8-145**) and *N*,*N*-disubstituted amides (**8-146**) are polar compounds of low volatility, yet some of them may participate in the aroma of non-acidic foods. Commonly occurring compounds are mainly amides derived from formic and acetic acids. For example, beer contains hundredths to tenths mg/kg *N*-methylformamide (**8-145**, $R = H$, $R^1 = CH_3$), *N*-methylacetamide (**8-145**, $R = R^1 = CH_3$), *N*,*N*′-dimethylacetamide (**8-146**, $R = R^1 = R^2 = CH_3$), *N*-(2-methylbutyl)acetamide, where $R = CH_3$, $R^1 = CH_2CH(CH_3)CH_2CH_3$, its isomer *N*-(3-methylbutyl)acetamide, $R^1 = CH_2CH_2CH(CH_3)_2$ and *N*-(2-phenylethyl)acetamide (**8-147**), which are produced by condensation of carboxylic acids with amines followed by decarboxylation (Figure 8.70). Many non-volatile *N*-substituted formamides and acetamides are also produced in the Maillard reaction. Decarboxylation of asparagine catalysed by decarboxylases yields 3-aminopropionamide (**8-148**), which may become a precursor of acrylamide (see Section 12.2.2).

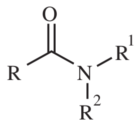
In addition to aliphatic amides, significant components of the aroma of shrimps, crabs, other marine crustaceans, molluscs and other marine animals include cyclic amides (lactams), such



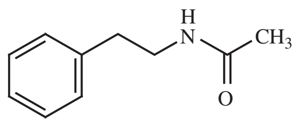
8-144, carboxylic acid amide



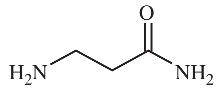
8-145, *N*-substituted carboxylic acid amide



8-146, *N*,*N*-disubstituted carboxylic acid amide

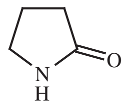


8-147, *N*-(2-phenylethyl)acetamide

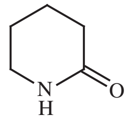


8-148, 3-aminopropionamide

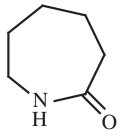
as 2-pyrrolidone (also known as γ-butyrolactam or butane-4-lactam, **8-149**), 2-piperidone (δ-valerolactam or pentane-5-lactam, **8-150**), 2-perhydroazepinone (ε-caprolactam or hexane-6-lactam, **8-151**) and their *N*-methyl-substituted analogues. Cyclic amides are formed by dehydration of the corresponding amino acids.



8-149, 2-pyrrolidone



8-150, 2-piperidone



8-151, 2-perhydroazepinone

As well as aspartic and glutamic acid amides (glutamine and asparagine), foods contain a number of other non-volatile amides derived almost exclusively from glutamine or glutamic acid. An example is the amino acid L-theanine (see Section 2.2.1.2.3) in tea leaves.

Substituted amides derived from amino acids (biogenic amines) and cinnamic acids are also frequent components. Amides derived from biogenic amines are constituents of cell walls of higher

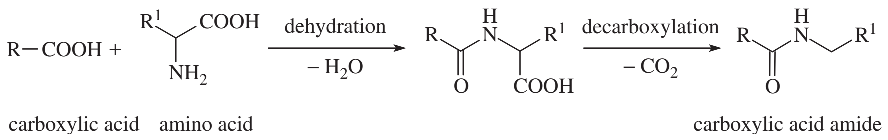
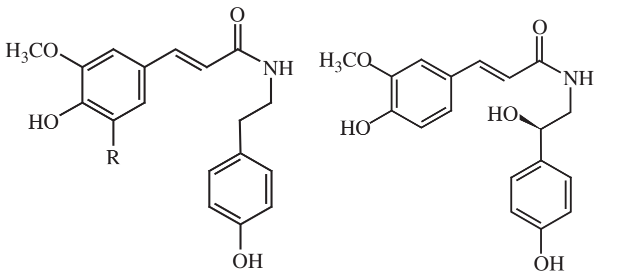


Figure 8.70 Formation of amides from carboxylic acids and amino acids.

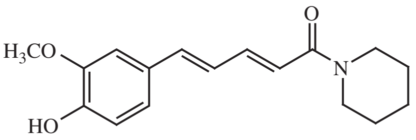
plants that are often classified as alkaloids. It is assumed that tyramine amides, such as (*E*)-*N*-sinapoyltyramine (**8-152**), act as a barrier against the penetration of pathogens in the plant cell walls, because they increase tissue rigidity and reduce its digestibility. Another amide derived from tyramine and ferulic acid (**8-152**), exhibiting antioxidant effects, occurs in black pepper. Some other amides are derived from dopamine, phenylethylamine and octopamine. For example, (*E*)-*N*-*p*-coumaroyloctopamine and (*E*)-*N*-feruloyloctopamine (**8-152**) occur in garlic skin as major antioxidants. Also active as an antioxidant is the amide derived from the pepper alkaloid piperine via opening of the 1,3-dioxol ring and methylation of one hydroxy group (**8-153**). Unlike piperine and other pepper alkaloids (see Section 10.3.2.1.2), this amide is odourless and does not cause a burning sensation.



N-feruloyltyramine, R = H
N-sinapoyltyramine, R = OCH_3

N-feruloyloctopamine, R = H
N-*p*-coumaroyloctopamine, R = OCH_3

8-152, cinnamic acids amides

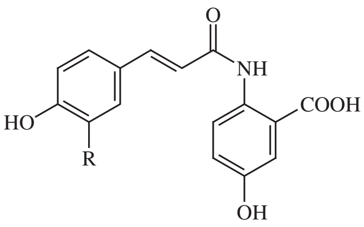


8-153, piperine derived amide with antioxidant properties

A group of about 40 amides derived from 5-hydroxyanthranilic or 5-hydroxy-4-methoxyanhranilic acids and cinnamic acids, trivially named avenanthramides, are important phytoalexins unique to oat grains (*Avena sativa*, Poaceae). The dominant compounds are derivatives of 5-hydroxyanthranilic acid: avenanthramide Bp derived from *p*-coumaric acid (also called avenanthramide A, **8-154**), avenanthramide Bc derived from caffeic acid (also called avenanthramide C) and avenanthramide Bf derived from ferulic acid (also called avenanthramide B). The main compound (40–132 mg/kg) is avenanthramide Bf (**8-154**). Approximately 6% of oat antioxidants are anthramides derived from caffeic acid.

Related amides are vanillylamides, such as capsaicin and dihydrocapsaicin, which are derived from vanillylamine and fatty acids. Vanillylamides are the main hot substances of peppers. A similar compound in peppers is capsaicinol, which does not create a burning sensation, but is effective as an antioxidant (see Section 10.3.2.1.7).

$^{\beta}N$-Alkanoyl-5-hydroxytryptamides occurring in the wax layer of coffee beens are derived from serotonin and $C_{22:0}$ to $C_{24:0}$ fatty acids. It has been shown that all these amides are responsible for the

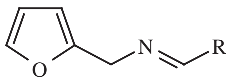


8-154, avenanthramide Bp, R = H
avenanthramide Bc, R = OH
avenanhramide Bf, R = OCH_3

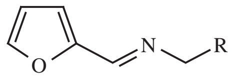
stomach irritation caused by stimulation of gastric juice secretion after ingestion of coffee brews.

8.2.10.2 Properties and reactions

Aldehydes and ketones react with ammonia and primary amines to form imines. Reaction with aldehydes yields aldimines, and ketimines arise in reactions with ketones (Figure 8.28). Imines derived from aliphatic carbonyl compounds are generally unstable and are transformed to more stable products, such as amines, diamines and others. Secondary amines react with aldehydes and ketones with the formation of enamines (Figure 8.29). Imines are similarly flavour-active compounds derived from furan-2-carbaldehyde. For example, the aromas of *N*-furfuryl(isobutylidene)amine, *N*-(furfuryl)isopentylideneamine (**8-155**) and *N*-(furfurylidene)isobutylamine (**8-156**) reportedly resemble chocolate.



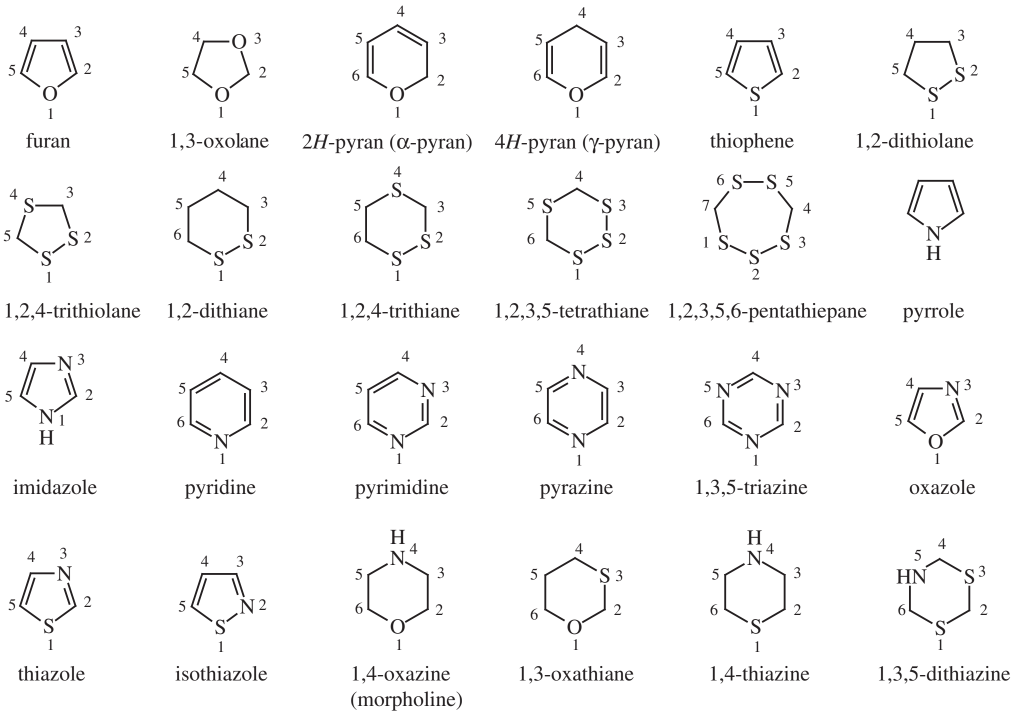
8-155, *N*-(isobutylidene)-2-furfurylmethylamine, R = $CH(CH_3)_2$



8-156, *N*-(furfurylidene)isobutylamine, R = $CH(CH_3)_2$
N-(furfurylidene)isopentylamine, R = $CH_2CH(CH_3)_2$

8.2.11 Heterocyclic compounds

Heterocyclic compounds are those in which one or more carbon atoms in the ring are replaced by another element, which is called a heteroatom. Most common in foods are five-membered and six-membered heterocyclic compounds containing as the heteroatom oxygen, sulfur or nitrogen. Also common are heterocycles containing more of the same or different heteroatoms. Heterocyclic compounds are important odour- and taste-active components of many foods. The basic structures of most oxygen, sulfur and nitrogen heterocycles are shown in formulae **8-157**. More important than these basic heterocycles, however, are their



8-157, structures of basic heterocyclic compounds

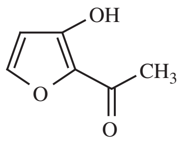
derivatives, particularly alkyl, acyl, hydroxy, oxo and certain others. A number of important aromatic compounds are derived from dihydro, tetrahydro or hexahydro derivatives of these heterocyclic compounds. Some heterocyclic compounds exhibit different physiological effects. Heterocycles containing polar functional groups are non-volatile, such as virtually all imidazole and pyrimidine derivatives, but they often have other functions in living organisms.

Heterocyclic compounds are formed mainly in non-enzymatic browning reactions, especially in food surface layers during the thermal processing of foods by baking, frying and roasting. They also arise by pyrolysis of proteins, carbohydrates, lipids and other food components; some heterocyclic compounds result from reactions catalysed by enzymes.

8.2.11.1 Classification, structure, terminology and occurrence

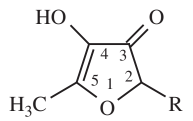
8.2.11.1.1 Furans

A large number of furan derivatives are produced in non-enzymatic browning reactions as dehydration products of carbohydrates and related compounds, such as ascorbic acid (γ-lactones can also be considered furan derivatives). Most furans are common to many thermally processed foods. Higher levels of these compounds are also present in acid protein hydrolysates. Furan-2-carbaldehyde has the characteristic pleasant woody aroma resembling nuts, which arises from pentoses and ascorbic acid, and 5-methylfuran-2-carbaldehyde, which is a product of 6-deoxyhexoses. A fatty herbal type flavour is provided by 5-hydroxymethylfuran-2-carbaldehyde arising from hexoses. 2-Acetylfuran arising from hexoses and pentoses is an important component of a number of foods, such as fruits, wine and beer, with a sweet, balsamic flavour. 2-Acetyl-3-hydroxyfuran known as isomaltol (**8-158**) has a caramel-like flavour. Isomaltol is formed from hexoses and 4-*O*-substituted glucoses at higher concentrations.



8-158, isomaltol

The most important furans in major food commodities are 4-hydroxy-2*H*-furan-3-ones (**8-159**), which are characterised by a planar arrangement of molecules and the same configuration of the enol, hydroxyl and oxo groups. Furanones occur as racemic mixtures because of their keto–enol tautomerism (Figure 8.71). Some other compounds have analogous structures as furanones, such as maltol, 3-hydroxy-2*H*-pyran-2-one and cyclopentenolones. All compounds with this structure resemble more or less the caramel



8-159, 4-hydroxy-5-methyl-2*H*-furan-3-one (norfuraneol), R = H
4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol), R = CH_3
2-ethyl-4-hydroxy-5-methyl-2*H*-furan-3-one (homofuraneol), R = CH_2CH_3

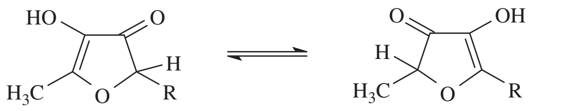
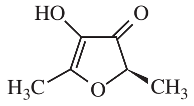


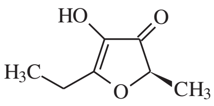
Figure 8.71 Structure of compounds with caramel flavour.

flavour with sugary and sweet notes; this is different, however, if the hydroxyl group is methylated or completely missing.

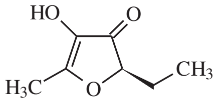
An important compound is 4-hydroxy-5-methyl-2*H*-furan-3-one, known as norfuraneol. Norfuraneol occurs in caramel, roasted chicory root and also in meat broth. 4-Hydroxy-2,5-dimethyl-2*H*-furan-3-one, known as furaneol, strawberry furanone or pineapple furanone, arises in the Maillard reaction from L-rhamnose (Figure 4.39) and in a reaction of methylglyoxal with hydroxyacetone (Figure 4.41). It occurs, for example, in strawberries, pineapple, roasted almonds, popcorn, meat broth and a number of other foods as a racemic mixture. The structure of (+)-(*R*)-furaneol responsible for the characteristic odour is given in formula **8-160**. The odour of furaneol is sugary, jammy and reminiscent of strawberries and, at higher concentrations, caramel (threshold concentration is 1–4 μg/l in air). Racemic homofuraneol exists in two tautomeric forms through the keto–enol isomerisation, as 5-ethyl-4-hydroxy-2-methyl-2*H*-furan-3-one (homofuraneol I) or 2-ethyl-4-hydroxy-5-methyl-2*H*-furan-3-one (homofuraneol II). Homofuraneol I and homofuraneol II occur in a ratio of 1 : 3 to 1 : 2, which are in equilibrium with each other. Structures of (+)-(*R*)-isomers are given in formulae **8-161** and **8-162**, respectively. Only one isomer, (*R*)-homofuraneol I was found to have a strong roasted sweet scent. The other isomers have sweet, less roasted odour. Homofuraneol is found, for example, in soy sauce.



8-160, (+)-(*R*)-furaneol



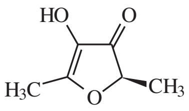
8-161, (+)-(*R*)-homofuraneol I



8-162, (+)-(*R*)-homofuraneol II

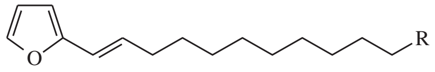
An example of a furanone with methylated hydroxyl in position C-4 is 4-methoxy-2,5-dimethyl-2*H*-furan-3-one, also known as mesifurane, which is derived from furaneol. (+)-(*R*)-Mesifurane (**8-163**) has a burnt and caramel-like odour, but is more subtle and mellow than the odour of furaneol. Mesifurane accompanies furaneol in fruits (such as pineapple, raspberry, strawberry and grape) and other foods. 2,5-Dimethyl-2*H*-furan-3-one, which does not contain the C-4 hydroxy group occurs as a constituent of bread and coffee flavour; its odour resembles bread.

Autoxidation of linoleic and linolenic acids yields 2-pentylfuran, isomeric 2-(pent-2-en-1-yl)-furans are produced from linolenic



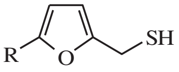
8-163, (+)-(*R*)-mesifurane I

acid with a specific type of rancidity called reversion. They, along with other oxidation products in soybean oil and some other oils, possess a green odour resembling beans, which is known as reversion flavour (see Section 3.8.1.10.1). Avocados (*Persea americana*, Lauraceae) and other *Persea* species contain a group of furans commonly referred to as avocadofurans that are substituted at C-2 with various saturated or unsaturated side chains. The first two avocadofurans identified from avocado fruit and seeds were 2-(tridec-12-yn-1-yl)furan (avocadodyenofuran) and 2-(tridec-12-en-1-yl)furan (avocadodienofuran) (**8-164**). Since then, many other avocadofurans with growth inhibitory and insecticidal activities have been identified.

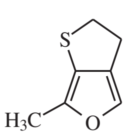


8-164, avocadienofuran, R = CH=CH$_2$
avocadynenofuran, R = C≡CH

Some furans containing a sulfur atom in the aliphatic side chain, and also some thienofurans, contribute significantly to the aroma of roasted coffee. Examples of compounds of the first group are 2-furanmethanethiol (furfuryl mercaptan), its 5-methyl derivative (**8-165**) and 2-methyl-3-furanthiol which are found in coffee. The presence of 2-furanmethanethiol and 2-methyl-3-furanthiol has also been reported in certain wines. On the other hand, 2-methyl-3-furanthiol can contribute to stored orange juice off-flavor. A representative of the second group of compounds is diterpene kahweofuran, also known by the systematic name of 2-methyl-3-oxa-8-thiabicyclo[3.3.0]octa-1,4-diene or 2,3-dihydro-6-methylthieno[2,3-*c*]furan (**8-166**), which is only found in roasted coffee. All these compounds have very low odour thresholds. A number of other furan derivatives containing sulfur in the molecule arise as thermal degradation products of thiamine, which is, therefore, used as an ingredient for simulating meat aroma, together with proteins, sugars (such as ribose) and sulfur amino acids (e.g. cysteine) in the so-called reaction flavours, also known as process flavours. Possibly the best known commercial



8-165, 2-furanmethanethiol, R = H
5-methyl-2-furanmethanethiol, R = CH$_3$

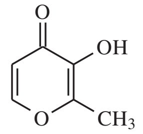


8-166, kahweofuran

reaction flavour is 2,5-dimethyl-3-hydroxy-2*H*-furan-3-one (furaneol), obtained by heating L-rhamnose with L-proline.

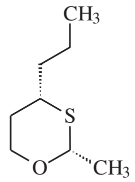
8.2.11.1.2 Pyrans

Pyrans occurring in foods are hypothetically derived from α-pyran or γ-pyran, or from α- or γ-pyrones, respectively. δ-Lactones can also be considered derivatives of α-pyran. An important α-pyrone is 3-hydroxypyran-2-one, also known as 3-hydroxy-2-pyrone or 3-hydroxy-2*H*-pyran-2-one, which is a δ-lactone of 2,5-dihydroxypenta-2,4-dienoic acid with a caramel-like flavour, which arises from ascorbic acid (see Section 5.14.6.2.2). Undoubtedly, the most important γ-pyrone is 3-hydroxy-2-methylpyran-4-one, which is known as maltol (**8-167**), featuring a caramel (or malt) smell and taste. A large number of other γ-pyrones are formed in non-enzymatic browning reactions, but as flavourings they are of little consequence.



8-167, maltol

Additionally, aroma components of some foods are six-membered heterocycles containing an oxygen atom and sulfur in the molecule. An example of such a compound is (2*R*,4*S*)-2-methyl-4-propyl-1,3-oxathiane (**8-168**), also known as *cis*-tropathiane, which occurs in the yellow passion fruit (*Passiflora edulis*, f. *flavicarpa*, Passifloraceae), pineapple (*Ananas comosus*, Bromeliaceae), whisky and white wines.



8-168, (2*R*,4*S*)-2-methyl-4-propyl-1,3-oxathiane

8.2.11.1.3 Thiophenes

Thiophenes are found in many foods and often contribute significantly to their organoleptic properties. They are important as aromatic components of meat, roasted coffee, roasted nuts, onions and other foods. For example, while unsubstituted thiophene has an odour reminiscent of benzene, 2-methyl substituted thiophenes positively affect the flavour of canned meat. 2-Methylthiophene odour is described as green, heated onion, sulfurous and sweet; dimethylthiophenes are important components of fried onion aroma, 2-acetyl-3-methylthiophene is reminiscent of honey and roasted nuts and 3-acetyl-2,5-dimethylthiophene has a sulfurous odour.

Thiophenes are formed by different reactions. The general reaction is that of furan derivatives with hydrogen sulfide (Figure 8.72). This mechanism is assumed, for example, in the formation of some thiophene derivatives from 4-hydroxy-2*H*-furan-3-ones. Alkylthiophenes substituted in positions C-2 and C-3 may arise in reactions of 2-mercaptoethanal with unsaturated aldehydes, such as acrolein or but-2-enal (Figure 8.73). 2-Mercaptoethanal is a product of Strecker degradation of cysteine.

The characteristic components of the fried onion aroma are 2,4-dimethylthiophene and 3,4-dimethylthiophene. They are produced from di(prop-1-en-1-yl)disulfide (Figure 8.74), which is formed from isoalliin via the corresponding thiosulfinate, in the same way as diallyl disulfide gives alliin via allicin.

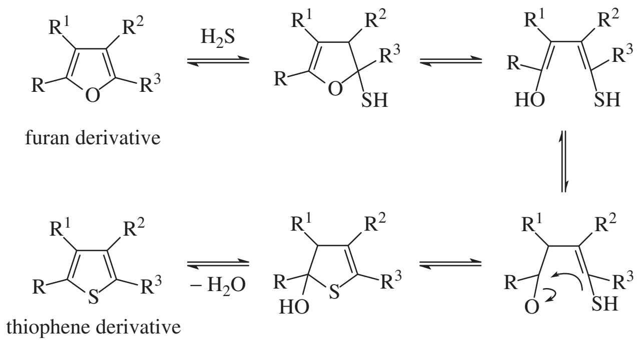


Figure 8.72 Formation of thiophenes from furans.

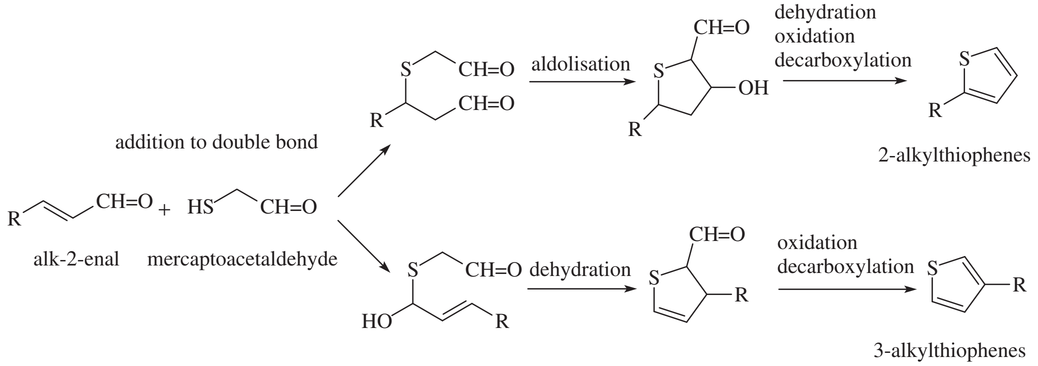


Figure 8.73 Formation of 2-alkylthiophenes and 3-alkylthiophenes.

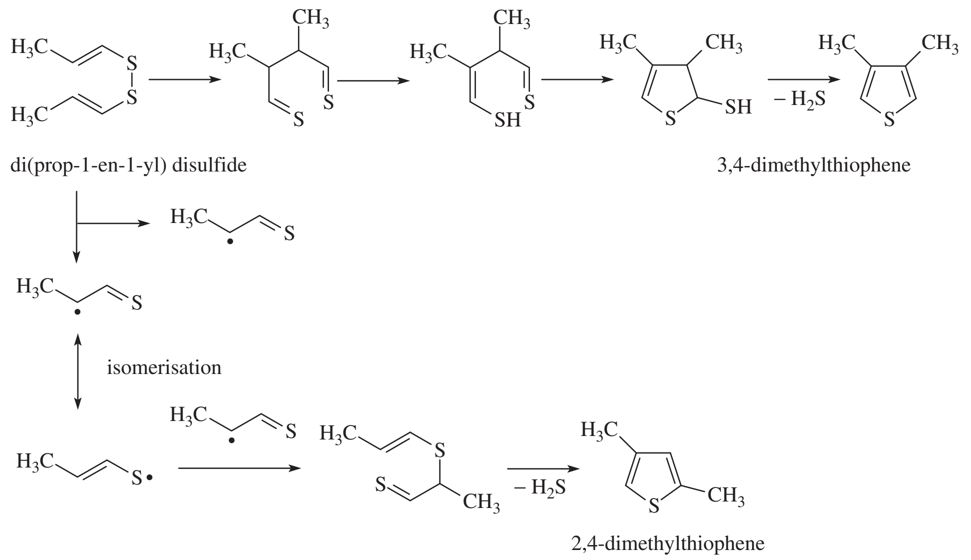


Figure 8.74 Formation of 2,4-dimethylthiophene and 3,4-dimethylthiophene in roasted onion.

Common compounds generated by the thermal processes are also thienothiophenes (**8-169**); these are, for example, components of roasted coffee volatiles.

thieno[2,3-*b*]-thiophene thieno[3,2-*b*]-thiophene

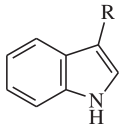
8-169, thienothiophenes

8.2.11.1.4 Pyrroles

Pyrroles generally have an intense and unpleasant odour, but the odour of some pyrroles and derived compounds resembles caramel, nuts, bread or chocolate upon dilution. A non-volatile pyrrole derivative is 5-oxopyrrolidine-2-carboxylic acid, produced by heating glutamic acid or glutamine.

Many pyrroles arise mainly as products of the Maillard reaction of carbohydrates and proline. Unsubstituted pyrrole and some alkylsubstituted pyrroles also result from pyrolysis of other amino acids. For example, pyrrole appears in the pyrolysate of glycine, serine, threonine or proline, 1-methylpyrrole arises from hydroxyproline, and 2-methylpyrrole and 3-ethyl-4-methylpyrrole are products of serine and threonine degradation. In general, pyrroles may arise in reactions of furans with ammonia (Figure 8.75), analogous to the reaction of furans with sulfane, which yields thiophenes. Pyrroles acylated in position C-2 of the nuclei are formed in reactions of amino acids with 2-acylfurans (Figure 8.76) and the corresponding pyridines are similarly produced. A particularly important compound is 2-acetyl-1-pyrroline, which is a characteristic aroma component of white bread crust, basmati rice and popcorn (see Section 8.2.12.4.1)

Indoles (4,5-benzopyrroles) are components of a generally undesirable odour of certain foods. Typical compounds are indole and 3-methylindole, which is trivially called skatole (**8-170**). At higher concentrations, these compounds have a faecal smell (as they are also found in faeces), but in low concentrations, they have a flowery smell. Both indoles are the result of degradation of tryptophan by microorganisms (also in the digestive tract of animals) or by pyrolysis of tryptophan. For example, these compounds contribute to a specific boar odour in pork meat and the typical odour of cooked beef tripe (see Section 8.2.12.1). For this reason, the traditional



8-170, indole, R = H
3-methylindole (skatole), R = CH_3

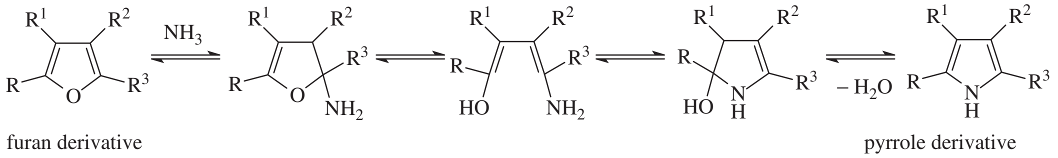


Figure 8.75 Formation of pyrroles from furans.

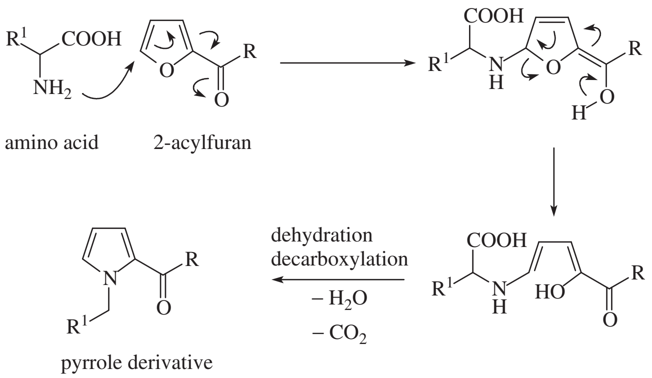


Figure 8.76 Formation of 2-acylpyrroles.

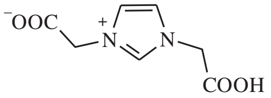
way of cooking tripe soup includes heating-up and removal of the first broth. The threshold concentration of skatole is very low (0.006 μg/l in water), and the acceptable concentration in pork fat is 0.15–0.25 mg/kg.

8.2.11.1.5 Imidazoles

Imidazoles are virtually non-volatile compounds and therefore do not act as odour-active substances. The main reaction leading to the formation of simple imidazoles is that of α-dicarbonyl compounds with aldehydes and ammonia (Figure 8.77). The basic member of the homologous series, unsubstituted imidazole (Figure 8.77, $R^1 = R^2 = R^3 = H$), thus arises from glyoxal, formaldehyde and ammonia, 4-methylimidazole (Figure 8.77, $R^1 = CH_3$, $R^2 = R^3 = H$) is analogously formed from methylglyoxal, formaldehyde and ammonia and the precursors of 2-methylimidazole (Figure 8.77, $R^1 = R^2 = H$, $R^3 = CH_3$) are glyoxal, acetaldehyde and ammonia. 4-Methylimidazole is found in large quantities (50–700 mg/kg) in caramel (see Section 4.7.6), when ammonia or ammonium salts are used as catalysts. Common dark beers and cola drinks may contain more than 250 μg/kg of 4-methylimidazole. It acts as a convulsant (an agent that causes convulsions) for some animals at very high doses and is a probable carcinogen; therefore in the European Union the legal limit was established at 250 mg/kg of caramel.

Similar effects were observed for 2-methylimidazole that is also found in caramel, together with polyhydroxy-substituted imidazoles that are produced from glycosuloses, 3-deoxyglycosuloses and ammonia. These imidazoles are unstable and at higher temperatures decompose to imidazole, its methyl derivatives and the corresponding glycols. An example of these compounds is 2-acetyl-4-(*arabino*-1,2,3,4-tetrahydroxybutyl)imidazole.

Non-volatile imidazoles that result from amino acids in reactions with α-dicarbonyl compounds, aldehydes and ammonia are betaines. The basic member of the homologous series of these imidazoles is 3-carboxymethyl-1-imidazolium ethanoate, which arises as the main product in the reaction of glycine with glyoxal and ammonia (**8-171**).



8-171, 3-carboxymethyl-1-imidazolium ethanoate

8.2.11.1.6 Pyridines

Descriptive terminologies for the odours of pyridines use terms such as green, bitter, astringent, roasted, burnt, pungent, solvent and fishy, none of which could be considered desirable. Their presence in some food commodities, such as beer and whisky, is disagreeable and associated with a cardboard, oxidised and harsh flavour. In roasted coffee, pyridines may contribute to a pleasant smell that is, however, less pleasant than the smell of pyrazines.

Some pyridines may arise, like thiophenes and pyrroles, in reactions of amino compounds with 2-acylfurans (Figure 8.78). Many pyridines are formed by thermal degradation of amino acids, proteins and other nitrogenous compounds. For example, a large number of pyridines present in roasted coffee result from the pyrolysis of alkaloid trigonelline. During the roasting process, trigonelline breaks down in two ways (Figure 8.79). The first pathway is the migration of the *N*-methyl group, which gives rise to methyl nicotinate (about 7%). Its content in roasted coffee is about 6 g/kg. In the presence of water, methyl nicotinate is hydrolysed to nicotinic acid. About 88% of trigonelline is decomposed via the second pathway (decarboxylation) to a number of pyridines. An important volatile product is unsubstituted pyridine, produced at a concentration of

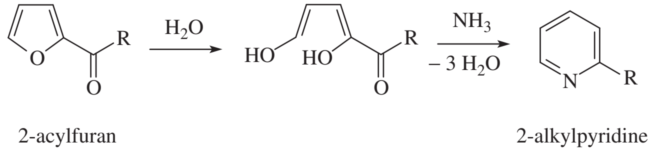


Figure 8.78 Formation of 2-alkylpyridines from 2-acylfurans and ammonia.

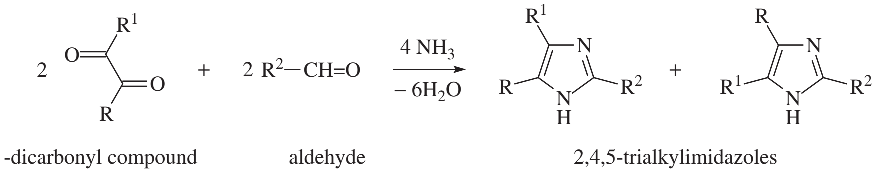


Figure 8.77 Formation of imidazoles from α-dicarbonyl compounds, aldehydes and ammonia.

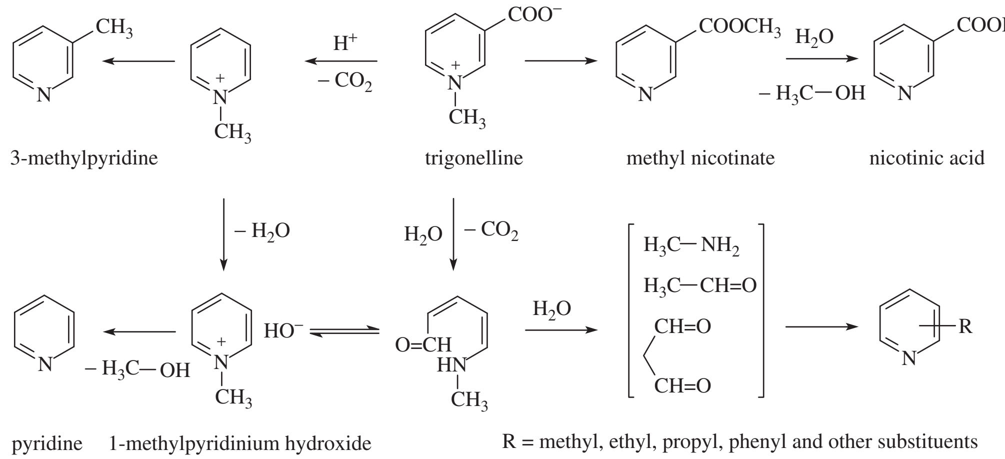
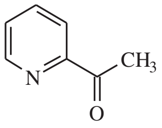


Figure 8.79 Main products of trigonelline pyrolysis.

about 200 mg/kg (5.3% of degraded trigonelline). Higher amounts of pyridine in coffee cause an unpleasant aroma. Another major product is 3-methylpyridine (0.25%). An important intermediate is considered to be 1-methylpyridinium hydroxide, which decomposes to give methylamine, acetaldehyde and malondialdehyde. The *N*-methylpyridinium ion shows chemopreventive effects. Reactions of these and certain carbonyl compounds and other fragments yield large quantities of different products, in particular various alkylpyridines and arylpyridines, of which 4-phenylpyridine, formed at a level of about 5 g/kg, is particularly significant.

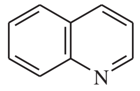
A significant alkyl pyridine is 2-pentylpyridine, which is found, for example, in meat. It may be formed by ring closure of deca-2,4-dienals by ammonia. Important components of cocoa, bread, aromatic rice, popcorn and other cereal products are 2-acetylpyridine (**8-172**) and 6-acetyl-1,2,3,4-tetrahydropyridine, which occurs in tautomeric equilibrium with 6-acetyl-2,3,4,5-tetrahydropyridine. Tetrahydropyridines develop together with 2-acetyl-1-pyrroline during the baking process (see Section 8.2.12.4.1).



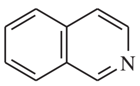
8-172, 2-acetylpyridine

Further flavour components of coffee (and other foods) are different quinolines (**8-173**) and isoquinolines (**8-174**), which result from trigonelline. Some pyridines (aminoimidazopyridines) and quinolines (aminoimidazoquinolines) produced in the Maillard reaction are classified as processing food contaminants (see Section 12.2.1).

Pyridines and pyrazines, such as 2-ethylpyridine, 2,3,5-trimethyl pyridine, 2-ethyl-3,5-dimethyl pyridine, 2,3-dimethylpyrazine and 2,5-dimethylpyrazine, are the most prominent classes of odorous compounds identified as being responsible for the odour of a cigar smoker's breath. They may be generated during cigar pyrrolysis by cleavage of nicotine or by Maillard reaction.



8-173, quinoline



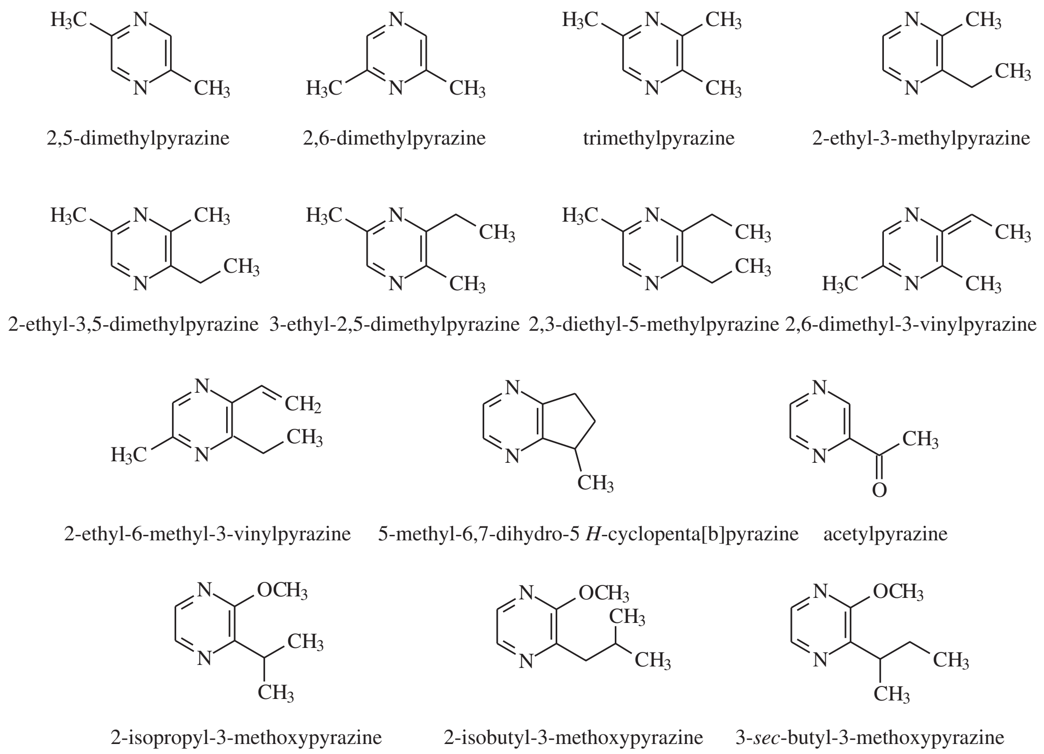
8-174, isoquinoline

8.2.11.1.7 Pyrazines

Pyrazine derivatives (alkylpyrazines, acylpyrazines, alkoxypyrazines and other derivatives, **8-175**) are present in virtually all heat processed foods (such as meat, bread, cocoa, roasted coffee and nuts), where they are the major carriers of the characteristic burnt, roasted and nutty odour. Pyrazines arise mostly as products of the Maillard reaction and by pyrolysis of some amino acids. 2-Alkyl-3-methoxypyrazines, which are odour components of various vegetables, are formed as primary odorous compounds through enzymatic reactions.

The general scheme of formation of pyrazines as byproducts of Strecker degradation of amino acids with α-dicarbonyl compounds is shown in Figure 8.80. For example, the main product of glyoxal reaction with amino acids is unsubstituted pyrazine, the reaction of amino acids with a mixture of glyoxal and methylglyoxal yields methylpyrazine and the reaction of methylglyoxal itself gives 2,5-dimethylpyrazine as the main reaction product. 2,5-Dimethylpyrazine is likewise produced by pyrolysis of threonine via the corresponding 2,5-dioxopyrazine. From simple alkylpyrazines, the methyl and ethyl substituted derivatives (Figure 8.81) are produced on reaction with formaldehyde and acetaldehyde, respectively. Recently an alternate route to pyrazine formation was proposed, which is based on dimerisation of azomethine ylids formed in the reaction of amino acids with 2-oxo acids. Simple alkylpyrazines may also form as pyrolysis products of non-volatile polyhydroxyalkyl-substituted pyrazines, which are reaction products of reducing sugars with amino acids. 2,3-Dimethylpyrazine and 2,5-dimethylpyrazine have also been identified as important odorous constituents responsible for the odor of a cigar smoker's breath.

Many pyrazines are synthesised and used to flavour foods, such as instant coffee, ice creams, potato products and many



8-175, selected important pyrazines

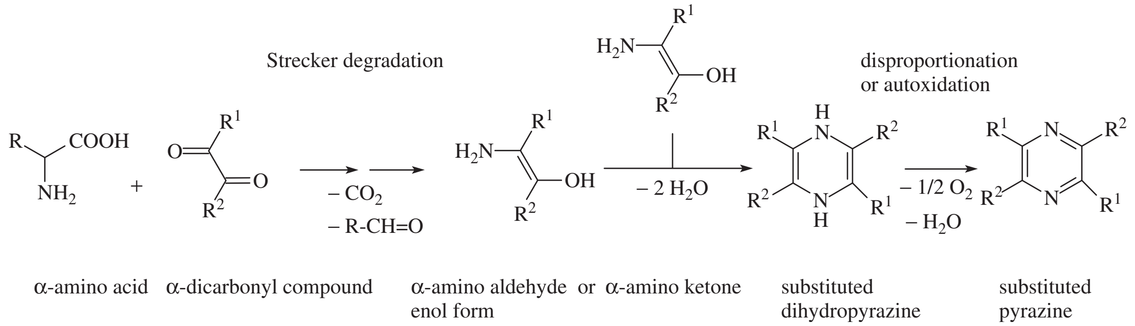


Figure 8.80 Formation of alkylpyrazines from α-dicarbonyl compounds and amino acids.

others. For example, the odour of 2,5-dimethylpyrazine resembles roasted hazelnuts, and 2,6-dimethylpyrazine and trimethylpyrazine have a chocolate-like odour. Important components of coffee aroma include a number of pyrazines, such as 2,3-diethyl-3-methylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,6-dimethyl-3-vinylpyrazine and 2-ethyl-6-methyl-3-vinylpyrazine. The flavour-active components of popcorn and white bread are acetylpyrazine, 2-ethyl-3-methylpyrazine, 3-ethyl-2,5-dimethylpyrazine and 5-methyl-6,7-dihydro-5*H*-cyclopenta[*b*]pyrazine. Cyclopentapyrazines, specifically 6,7-dihydro-5*H*-cyclopenta[*b*]pyrazines, are formed in reactions of cyclopentenolones with aminoketones and ammonia.

In some plants and microorganisms, tetramethylpyrazine occurs as a product of metabolism. 2-Alkyl-3-methoxypyrazines are important odorous components of many types of vegetables. For

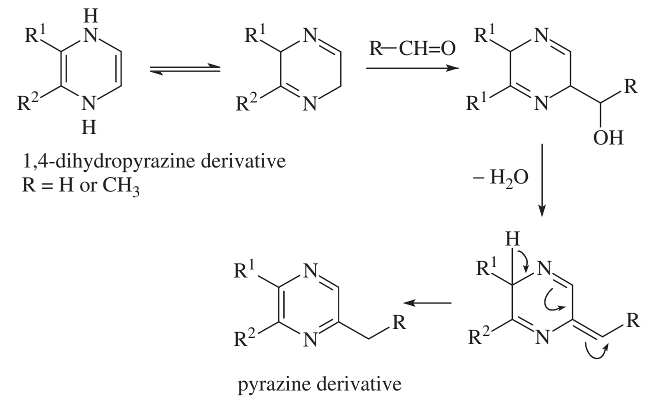
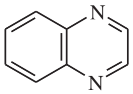


Figure 8.81 Formation of methylsubstituted and ethylsubstituted pyrazines.

the aroma of parsley, peas, peanuts or potatoes, 2-isopropyl-3-methoxypyrazine is an important compound, for the aroma of pepper 2-isobutyl-3-methoxypyrazine and for the aroma of parsley, peas and salad beet 2-*sec*-butyl-3-methoxypyrazine. 2-Alkyl-3-methoxypyrazines are also significant substances in the varietal character of wines, such as Cabernet Sauvignon and Sauvignon Blanc. The predominant compound is 2-isobutyl-3-methoxypyrazine, which occurs in these wines in concentrations of 3–56 ng/l, while its odour threshold concentration is 1 ng/l. Its content depends on the degree of maturation of grapes and with advancing maturity its concentration decreases and increases in grapes grown in a cool climate and low levels of sunlight. On the other hand, 2-isopropyl-3-methoxypyrazine has been suggested to be one of the key compounds responsible for the off-flavor produced by Asian lady beetle (*Harmonia axyridis*) in Frontenac and Leon Millot wines produced in Minnesota, and 2-methoxy-3,5-dimethylpyrazine was found to be responsible for the unpleasant, musty and moldy aroma of wine corks in Australian wines, which is similar to that produced by 2,4,6-trichloroanisole.

Thermal reactions, such as coffee roasting, also produce various quinoxaline (benzopyrazine) derivatives (**8-176**). Other quinoxalines (aminoimidazoquinoxalines) that arise in the Maillard reaction are classified as processing contaminants (see Section 12.2.1).



8-176, quinoxaline

8.2.11.1.8 Oxazoles

From the olfactive point of view, an unsubstituted oxazole odour is reminiscent of pyridine; alkylsubstituted oxazoles resemble melons with very ripe kiwi notes and with other more or less aggressive nuances and pungent notes. Oxazolidines, oxazolines and oxazoles (like other heterocycles) arise as reaction products of amino acids and reducing sugars, especially in the Maillard reaction, and occur as aroma components of roasted coffee, cacao, meat and other commodities. An important reaction is the Strecker degradation of cysteine. Formation of alkyloxazoles can be explained by condensation of aminoketones (products of the Strecker degradation of amino acids with α-dicarbonyl compounds) or hydroxyketones and ammonia with aldehydes via oxazolines that are oxidised to oxazoles with the elimination of water (Figure 8.82). For example, 4,5-dimethyloxazole (Figure 8.82, $R^1 = R^2 = CH_3$, $R^3 = H$) results from biacetyl and glycine (precursor of formaldehyde) and in an even greater amount as a product of formaldehyde and 2-aminobutan-3-one, which is a product of the Strecker degradation of amino acids with biacetyl. 2,4,5-Trimethyloxazole (Figure 8.82, $R^1 = R^2 = R^3 = CH_3$) arises from 2-aminobutan-3-one and acetaldehyde, 2,5-dimethyl-4-ethyloxazole (Figure 8.82, $R^1 = CH_2CH_3$, $R^2 = R^3 = CH_3$) and 2,4-dimethyl-5-ethyloxazole (Figure 8.82, $R^1 = R^3 = CH_3$, $R^2 = CH_2CH_3$) are products of pentane-2,3-dione and acetaldehyde and 2,4,5-trimethyl-3-oxazoline is formed as a reaction product of acetoin, acetaldehyde and ammonia (Figure 8.83). Analogously, 2-acetyloxazole is produced from serine and methylglyoxal.

8.2.11.1.9 Thiazoles and other sulfur heterocycles

Thiazole itself is smelling like pyridine, but substituted thiazoles, such as 2-acetylthiazole and 2,4-dimethyl-5-vinylthiazole, generally have a desirable odour, frequently described as nutty, green, roasted and vegetable-like. For example, the Strecker degradation of cysteine by methylglyoxal yields 2-acetylthiazole in thermally processed foods (Figure 8.83). A possible intermediate of this reaction is 2-acetyl-2-thiazoline with an intense aroma of fresh bread crust, which is similar to the odour of 2-acetyl-1-pyrroline. 2-Acetyl-2-thiazoline has been identified among the volatiles of beef broth and was reported as being an important component of roasted beef (14–28 μg/kg) and other foods. The reaction pathways leading to 2-acetyl-2-thiazoline involve the cysteine decarboxylation product cysteamine and the participation of sugar degradation products, such as methylglyoxal. The reaction mechanism (Figure 8.84) involves the condensation of cysteamine with methylglyoxal, isomerisation of the condensation product, its cyclisation to 2-(1-hydroxyethyl)-4,5-dihydrothiazole and isomerisation to 2-(1-hydroxyethylene)thiazolidine, which is oxidised by atmospheric oxygen in the presence of transition metals via hydroperoxide (with elimination of hydrogen peroxide) to 2-acetyl-2-thiazoline. Alternative mechanisms of oxidation of 2-(1-hydroxyethylene)thiazolidine (or 2-acetylthiazolidine) have also been suggested. In addition to the pathway involving methylglyoxal, another suggested pathway starts with cysteamine and 4-deoxy-D-*glycero*-hexo-2,3-diulose, which provides, on elimination of formaldehyde and glycolaldehyde, the necessary three carbons for the formation of 2-acetyl-2-thiazoline.

2-Methylthiazole and particularly 2-isobutylthiazole have an odour resembling vegetables, with the latter typically having the smell of fresh tomatoes and tomato leaves. Its biosynthesis requires leucine and cysteine (Figure 8.85). Related 2-isopropyl-4-methylthiazole (**8-177**), synthesised from cysteine and valine, is an odorous component of durian (*Durio zibethinus*, Bombacaceae).

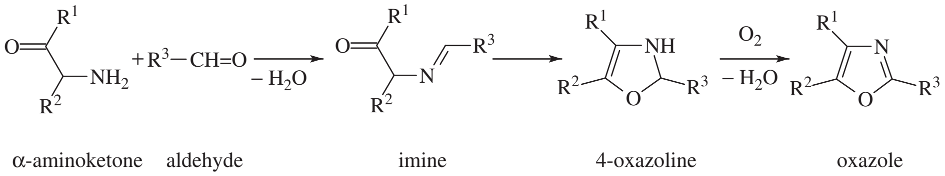


Figure 8.82 Formation of alkyloxazoles.

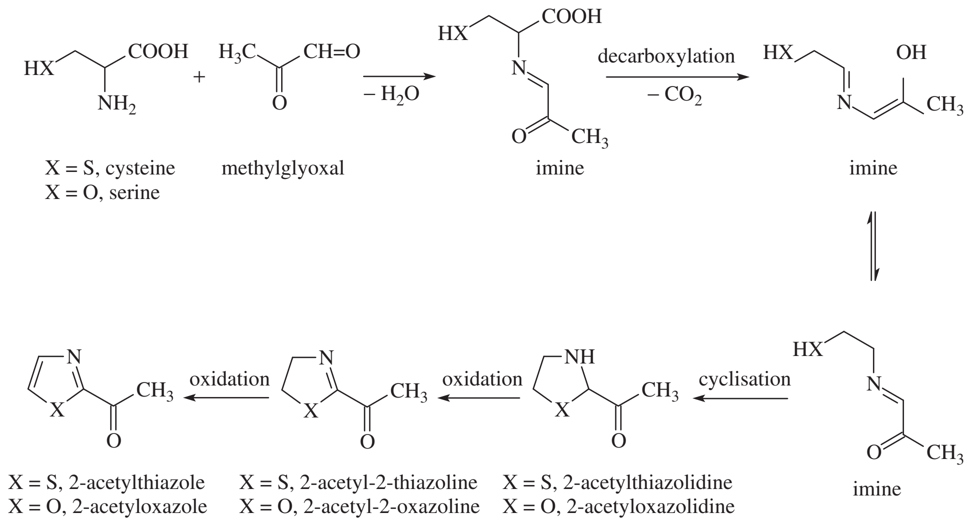


Figure 8.83 Formation of 2-acetyloxazole and 2-acetylthiazole.

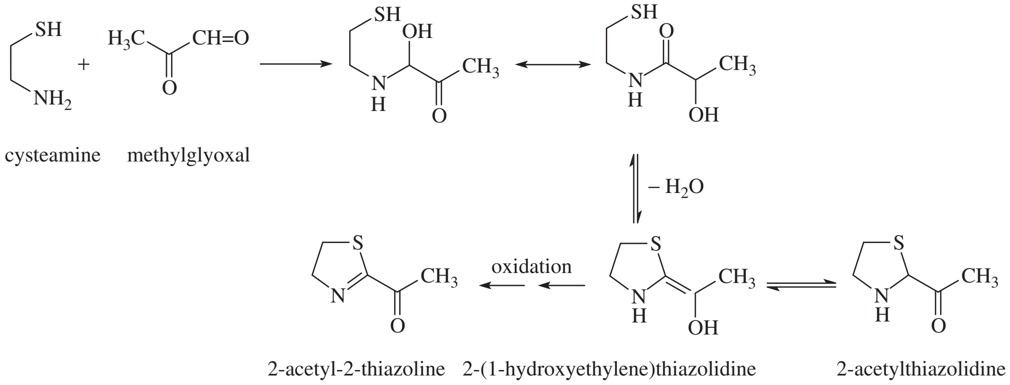


Figure 8.84 Formation of 2-acetyl-2-thiazoline.

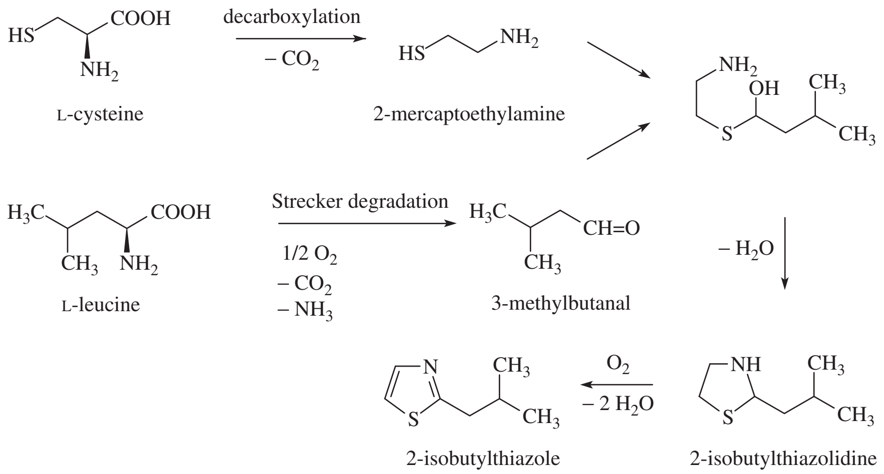
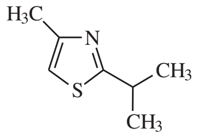


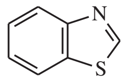
Figure 8.85 Biosynthesis of 2-isobutylthiazole.

Some thiazoles arise from degradation of other sulfur-containing compounds. An example of these thiazoles is non-volatile 4-methyl-5-(2-hydroxyethyl)thiazole that is formed by degradation of thiamine either by the action of thiaminases or by non-enzymatic reactions. Its dehydration, however, yields 4-methyl-5-vinylthiazole, which is a constituent of cocoa aroma.



8-177, 2-isopropyl-4-methylthiazole

Thiazoles are substances found in many thermally processed foods. They are present in coffee, boiled meat and boiled potatoes in high concentrations. A number of thiazoles can be synthesised and used to flavour various foods. Thiazoles are also carriers of undesirable odours and tastes, such as 2-acetylthiazole in beer, 2-acetyl-2-thiazoline (Figure 8.83) or benzothiazole (**8-178**) in condensed and powdered milk.



8-178, benzothiazole

8.2.11.2 Properties and reactions

Reactions of heterocyclic compounds are very complex. Significant reactions are discussed in the chapters dealing with the individual heterocyclic compounds; other important reactions are related to the Maillard reaction.

8.2.12 Aromatic substances of foods

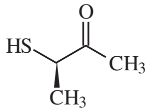
8.2.12.1 Meat and meat products

Hundreds of compounds contribute to the taste and odour of meat. These include non-volatile substances, which mainly affect the taste, and volatile substances, which particularly affect the odour. Raw meat has only a weak, faint odour, and aromatic substances are mainly the result of thermal processing of various precursors, largely from water-soluble compounds found in raw meat. Many of these precursors are formed during aging of meat due to important chemical, structural and functional changes in tissues. The characteristic flavour of cooked meat derives from thermally induced reactions occurring during heating, principally the Maillard reaction and degradation of lipids. Aroma character depends on the type of meat (pork, beef, mutton and chicken, each having their own different, distinctive odour) and the method of heat treatment (e.g. cooking, baking, frying or grilling), which is related to the water and fat content in the material and the temperature during the treatment. Parameters such as diet, sex, age, differences between muscles and storage conditions also play a significant role in determining the overall flavour sensation. For example, because of differences in the digestive systems between ruminants and non-ruminants, pork meat typically has more linoleic acid than beef or lamb, and pork and poultry meat contain higher amounts of polyunsaturated fatty acids than beef and lamb.

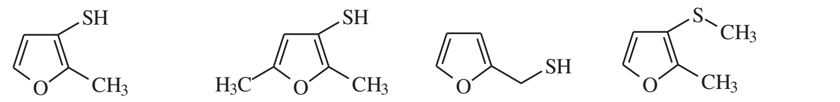
Lipid oxidation products and their reaction products with amino acids (proteins) have a considerable influence on the typical odour and taste of meat. Particularly significant aminocarboxylic acids include glutamic acid, alanine, threonine and lysine, guanidine compounds (creatine and creatinine), quaternary ammonium compounds (choline and carnitine), peptides (β-alanylhistidine peptides and some products of proteolysis), free nucleotides, nucleosides and their bases (especially inosine 5′-monophosphate, IMP), proteins, carboxylic acids (especially lactic acid), sugars (mainly glucose, fructose and their phosphates, ribose formed by hydrolysis of free nucleotides) and some vitamins (especially thiamine). Some of these compounds, such as glutamic acid and IMP, are additionally used as food additives, namely as flavour enhancers.

Another important reaction that leads to flavour-active compounds in meat is the Maillard reaction and the Strecker degradation of amino acids in particular. Many secondary compounds are formed by reactions of primary degradation products (hydrogen sulfide, methanethiol and ammonia) with Strecker aldehydes and carbonyl compounds being formed from lipid oxidation. Active aromatic substances include numerous alcohols, aldehydes, ketones, carboxylic acids, esters, lactones, various aliphatic sulfur compounds and oxygen, sulfur and nitrogen heterocycles. Not all volatile compounds are equally important to the meat aroma, but the resulting flavour is always determined by a relatively large number of different compounds.

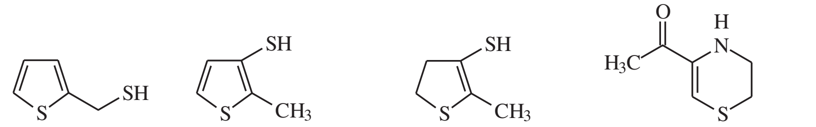
Particularly important substances for the basic flavour of baked and cooked meat are aliphatic thiols (such as methanethiol), sulfides (such as dimethyldisulfide, dimethytrisulfide and dimethyltetrasulfide), aldehydes (such as acetaldehyde, 2-methylpropanal and 3-methylbutanal), furans, pyridines and thiophenes with a mercapto group in position C-3 and their corresponding disulfides and some other aliphatic and heterocyclic sulfur compounds. Examples of important aliphatic thiols are 3-mercaptobutan-2-one (**8-179**) and 3-mercaptopentan-2-one found in cooked beef. A mixture of 3-mercapto-2-methylpentane-1-ol diastereoisomers (Figure 8.86) has a broth-like, sweaty and leek-like flavour. Very low odour threshold concentrations and an odour reminiscent of roasted meat are found in 2-methylfuran-3-thiol (**8-180**), which also occurs in beef broth, roasted coffee and other foods, 2,5-dimethylfuran-3-thiol, their corresponding disulfides and 2-furanmethanethiol (furfuryl mercaptan). The typical aroma of roast beef is found in 2-methylthiophene-3-thiol and 4,5-dihydro-2-methylthiophene-3-thiol. Important sulfur compounds in beef are 2-methyl-3-methylthiofuran, along with the other heterocyclic compounds 2-pentylpyridine, thiophene-2-thiol and 5-acetyl-2,3-dihydro-1,4-thiazine. Significant odorous components of heat-treated beef also



8-179, (*R*)-3-mercaptobutan-2-one



2-methylfuran-3-thiol 2,5-dimethylfuran-3-thiol 2-furfurylmethyl mercaptan 2-methyl-3-methylthiofuran



2-thienylmethyl thiol 2-methylthiophene-3-thiol 2.3-dihydro-5-methyl-thiophene-4-thiol 5-acetyl-2,3-dihydro-1, 4-thiazine

8-180, selected heterocyclic odorous substances in meat

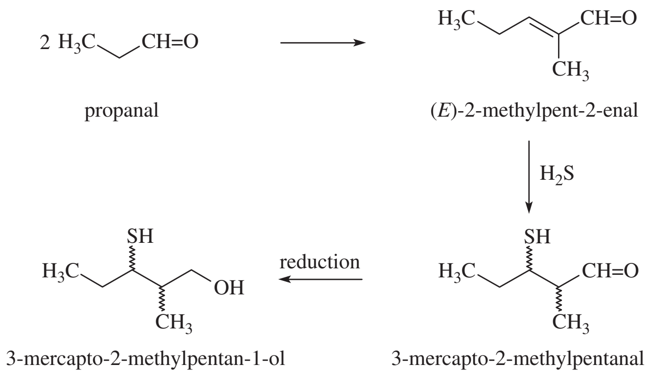
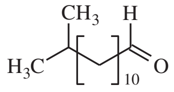


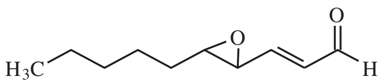
Figure 8.86 Formation of 3-mercapto-2-methylpentan-1-ol from propanal and hydrogen sulfide.

include 2-acetyl-2-pyrroline, which is otherwise an important component of bread crust flavour, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol), its lower homologue 4-hydroxy-5-methyl-2*H*-furan-3-one (norfuraneol), lactone 3-hydroxy-4,5-dimethyl-5*H*-furan-2-one (sotolon).

Some compounds formed from meat lipids have an important role. Such compounds include some carbonyl compounds, such as oct-1-en-3-one, hexanal, octanal, nonanal, (*Z*)- and (*E*)-non-2-enal, (2*E*,6*Z*)-nona-2,6-dienal, (2*E*,4*Z*)-deca-2,4-dienal and 12-methyltridecanal (**8-181**). Other important fatty acid oxidation products include (*E*)-4,5-epoxydec-2-enal (**8-182**), (*Z*)-octa-1,5-dien-3-one, (2*E*,6*Z*)-nona-2,6-dienal. Also important



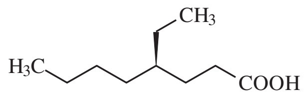
8-181, 12-methyltridecanal



8-182, (*E*)-4,5-epoxydec-2-enal

are higher thiols, 2-alkylpyridines, 2-alkyl-3-formylthiophenes and dihydrothiophenes, 2-alkylthiazoles, 2-alkyl-4-methylthiazoles, 2-alkyl-4,5-dimethylthiazoles, 5-alkyl-4-ethylthiazoles and 2-alkyl-4,5-diethylthiazoles. The alkyl is mostly pentadecyl derived from hexadecanal bound in plasmalogenes. Plasmalogenes also produce aldehyde 12-methyltridecanal (**8-181**) with the aroma of beef stew. The list of sensory active compounds is far from complete and many components of meat flavour are yet unknown.

For example, lamb/mutton has a distinctive aroma, which to some extent determines its lesser popularity. The main aromatic component of heat-treated meat is an unusual, branched fatty acid 4-ethyloctanoic acid (**8-183**) that has a waxy, fatty, creamy, moldy, sour sweaty, cheesy odour with animal-like nuances, and occurs as an almost racemic mixture of the enantiomers. The odour thresholds of the (+)-(*R*)- and (−)-(*S*)-enantiomers are <13 and <6 μg/l in air, respectively. 4-Ethyloctanoic acid (also identified in stewed beef gravy and goat and sheep cheeses) is accompanied by 4-methyloctanoic acid. The 4-ethyloctanoic acid content is approximately the same in raw meat but higher in fat.

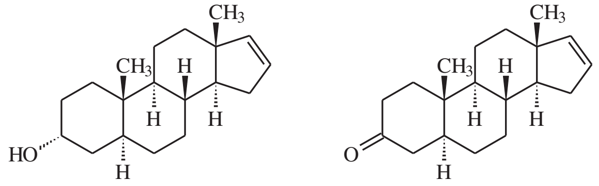


8-183, (*R*)-4-ethyloctanoic acid

For example, in dry-fermented and mold-ripened sausages, such as the Hungarian-type salami, the key aroma compounds are 2-methoxyphenol, eugenol, (*E*)-isoeugenol, 5-methyl-2-methoxyphenol, 4-propyl-2-methoxyphenol, 4-ethyl-2-methoxyphenol and 3-ethylphenol, phenylacetaldehyde, methional, acetic acid and 3-methylbutanoic acid.

A boar taint in processed pork is caused by the presence of the hormone pregnenolone and its metabolic products (**8-184**), which may similarly occur in meat of castrates and sows. The steroid alcohol (+)-(3α,5α)-androst-16-en-3-ol has a musk aroma, the corresponding oxidation product (+)-(5α)-androst-16-en-3-one has a smell resembling urine. A characteristic unpleasant odour then develops during the cooking of meat. Other compounds contributing to the boar taint are skatole, an intestinal degradation

product of tryptofan, which exhibits a faecal-like odor, and 4-phenylbut-3-en-2-one. The source of 4-phenylbut-3-en-2-one is not clear, but its seems to be related to cinnamic acid metabolism and with phenylalanine degradation.



(3α,5α)-androst-16-en-3-ol (5α)-androst-16-en-3-one

8-184, derivatives of androst-16-ene

8.2.12.1.1 Poultry and fish

The volatile components of raw chicken breast muscle include mainly carbonyls, thiols, sulfides and alcohols. The major volatile components of fried chicken are similar to volatiles of the meat of farm animals (e.g. common aldehydes, ketones, hydrocarbons and other compounds). Sulfur-containing compounds generated during thermal processing are also important for the flavour of chicken meat. Saturated and unsaturated aldehydes are very important – these are produced easily by oxidation of lipids and manifest negatively in stored poultry meat, which quickly becomes rancid. Feed plays an important role in imparting certain flavour characteristics to poultry meat.

The characteristic essential flavour-active components of fish and other aquatic animals are amines and other nitrogenous compounds. Trimethylamine arises by reduction of sensorially indifferent trimethylamine oxide (acting in the regulation of osmotic pressure in cells) in the tissue post mortem. The amount of trimethylamine oxide, as well as the amount of a number of simultaneously produced biogenic amines, depends primarily on the species, type and time of storage. Its content in fresh water fish is about 5 mg/kg, and in seafood is 40–120 mg/kg. Other important compounds are dimethylamine and ammonia.

Flavour-active components of fresh fish are further oxidation products of unsaturated fatty acids formed by the action of lipoxygenases. Important compounds are mainly alcohols and carbonyl compounds. For example, oxidation of eicosapentaenoic acid via 12-hydroperoxide yields (3*Z*,6*Z*)-nona-3,6-dienal, (2*E*,6*Z*)-nona-2,6-dienal, (3*Z*,6*Z*)-nona-3,6-dien-1-ol, (*Z*)-octa-1,5-dien-3-ol and (*Z*)-octa-1,5-dien-3-one, while the products of 15-hydroperoxide decomposition include (*Z*)-hex-3-enal, (*E*)-hex-2-enal, (*Z*)-hex-3-en-1-ol and pent-1-en-3-ol.

The fishy and greasy off-flavour appearing in long-term refrigerated storage of fish is almost entirely caused by lipid oxidation products that are present in larger quantities than in fresh fish. The important components are mainly (*Z*)-hex-3-enal, (*Z*)-hept-4-enal, (*Z*)-octa-1,5-dien-3-one and (3*Z*,6*Z*)-nona-3,6-dienal. An chief component is methional, the product of the Strecker degradation of methionine.

8.2.12.2 Milk and dairy products

8.2.12.2.1 Milk

Raw or gently pasteurised milk (e.g. for 10 seconds at 73 °C) has a fine characteristic odour and sweet taste. Typical components present in low concentrations are dimethylsulfide, biacetyl, 2-methylbutan-1-ol, (*Z*)-hept-4-enal and (*E*)-non-2-enal. Milk pasteurised at higher temperatures and Ultra High Temperature (UHT) milk present the so-called cooked flavour, the appearance of which is the first measurable manifestation of the chemical changes that occur in heated milk. The substances responsible for the cooked off-flavour are sulfane and other sulfur compounds. Of particular importance are dimethylsulfide, dimethyldisulfide and dimethyltrisulfide that are produced from proteins contained in the membranes of fat particles and from thiamine. Also relevant are alkane-2-ones (methylketones) generated by thermal decarboxylation of β-oxocarboxylic acids (mainly hexane-2-one, heptane-2-one and nonane-2-one), γ-lactones and δ-lactones produced by dehydration of γ- and δ-hydroxycarboxylic acids (mainly δ-decalactone and γ- and δ-dodecalactones). Important carbonyl compounds include biacetyl, hexanal, 3-methylbutanal, (*Z*)-hept-4-enal and (*E*)-non-2-enal. In the more intensive thermal treatment of milk (sterilisation), products of the Maillard reaction play a role, such as maltol and isomaltol, 5-hydroxymethylfuran-2-carbaldehyde, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) and 2,5-dimethylpyrazine.

Evaporation and drying leads to more extensive reactions than in UHT milk that include degradation of proteins and thiamine, Maillard reaction between proteins (preferably in bound lysine) and lactose, degradation of oxo- and hydroxycarboxylic acids and oxidation of fatty acids. Phospholipids of fat particles are particularly susceptible to oxidation. It is therefore mainly alkane-2-ones, lactones and the Maillard reaction products that are responsible for the odour of condensed and powdered milk. In addition to these compounds, typical aromatic substances are benzaldehyde, acetophenone, 2-aminoacetophenone, furfuryl alcohol and benzothiazole.

Flavour defects of milk and dairy products may be due to different causes. Raw or pasteurised milk products can pass into milk from unsuitable feed, or milk may absorb flavours from the surroundings during storage and distribution.

Chemical defects can occur in both raw and pasteurised milk as endogenous aromatic substances are formed by both enzymatic and chemical reactions. Oxidation of lipids is the major problem during storage of dried milk. Some of the oxidation products of lipids present in larger quantities play a negative role. For example, (*Z*)-hept-4-enal, oct-1-en-3-one and hexanal are important carriers of a full, creamy flavour, but at higher concentrations they confer cardboard, metal and green flavours. Long-term storage of condensed milk is reflected as old flavour, which is due to higher concentrations of normal compounds. Examples are benzothiazole and 2-aminoacetophenone (at a concentration >1 μg/kg) produced by decomposition of tryptophan. Often confused with oxidised flavour is the so-called sunlight flavour (characterised as burnt-protein or medicinal-like) that develops in unprotected milk stored in the light. Photooxidation activates riboflavin, which is

responsible for catalysing the conversion of methionine into sulfur compounds via methional.

Bacterial off-flavours result from the growth of psychrophilic bacteria that are present in milk due to poor sanitation or milk handling practices. Fruit odour is caused by carboxylic acid esters produced by bacteria *Pseudomonas fragii*, and a malt smell (caused by the presence of methylbutanal, 2-methylbutanal and 2-methylpropanal) is produced by bacteria *Streptococcus lactis* var. *maltigenes* and a phenolic odour by bacteria *Bacillus circulans*. Rancid smell, caused by lower fatty acids from butyric to lauric acids, is manifested as a result of lipolysis by milk lipase or bacterial lipases.

8.2.12.2.2 Cream and butter

For cream flavour, the most important components are lactones of hydroxycarboxylic acids, in particular, δ-decalactone, δ-dodecalactone and (*Z*)-dodec-6-eno-γ-lactone, to a lesser extent many other lactones, such as δ-tetradeca-, δ-hexadeca-, γ-tetradeca-, γ-hexadeca-, γ- and δ-octadeca- and γ- and δ-eicosalactones and other compounds (methanethiol, skatole and others).

The aroma of butter made from sweet cream is affected primarily by free fatty acids (especially capric and lauric acids), δ- and γ-lactones, dimethylsulfide, (*Z*)-hept-4-enal and the degradation products of tryptophan (indole and skatole). The butter obtained from sour cream contains mainly metabolic products of microorganisms (so-called starter cultures). Especially important compounds are biacetyl, lactic and acetic acids.

Lipases in stored butter gradually release fatty acids from triacylglycerols, and their presence can be detected as a rancid and soapy flavour when they reach 30–40% of their threshold concentrations, which is a result of their synergism. The reaction is called hydrolytic rancidity. Responsibe for the rancid flavour is mainly butyric acid, followed by caproic acid. Caprylic acid has a rancid soap-like flavour, capric and lauric acids only have soapy flavours. Odour (and taste) threshold concentrations in butter made from sweet cream are 50 (60) mg/kg for butyric acid, 85 (105) mg/kg for caproic acid, 200 (120) mg/kg for caprylic acid, >400 (90) mg/kg for caprinic (capric) acid and >400 (130) mg/kg for lauric acid, respectively. In long term stored butter, active oxidative rancidity products are (*E*)-non-2-enal, (*Z*)-non-2-enal in particular, while less active products are (*Z*)-hept-4-enal, oct-1-en-3-one and others. The rancid and soapy odour in butter can also be caused by contamination with anion active detergents, such as natriumdodecyl sulfate.

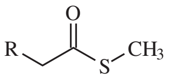
8.2.12.2.3 Fermented dairy products

Characteristic flavouring substances of fermented dairy products are metabolites of lactic acid bacteria, especially biacetyl, acetaldehyde, dimethylsulfide, lactic and acetic acids, various aldehydes, ketones and esters. An important product is carbon dioxide. The acetaldehyde content in good quality yoghurts is 13–16 μg/kg, while the biacetyl content is about four times higher.

8.2.12.2.4 Cheeses

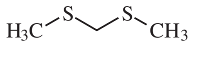
Cheeses contain large amounts of aromatic compounds that differ qualitatively and quantitatively according to types of cheeses. Important components of hard cheeses (Gouda type) include some carboxylic acid esters (ethyl butanoate, ethyl hexanoate), as well as carboxylic acids (acetic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric and caproic acids). Cheeses manufactured using bacteria of the genus *Propionibacterium* (such as Emmental and Gruyère) contain propionic acid and other lower fatty acids, methyl thioacetate, some oxocarboxylic acids, various alcohols, esters (such as ethyl butanoate), lactones (such as δ-decalactone), amines and other basic compounds (also skatole in addition to aliphatic amines), alkylpyrazines (e.g. 2-sec-butyl-3-methoxypyrazine), 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol), 2-ethyl-4-hydroxy-5-methyl-2*H*-furan-3-one (homofuraneol) and a range of other compounds.

Cheeses with a very pungent aroma caused by bacteria that live in the rind and on the cheese surface (such as Pont l'Eveque, Limburger or Romadur) contain phenol, cresol, acetophenone and methylthioesters of lower fatty acids (**8-185**), such as methyl thioacetate (R = H), methyl thiopropionate (R = CH_3) and methyl thiobutyrate (R = CH_2CH_3) as flavourings. Other important flavouring substances include C_4–C_{10} fatty acids, especially capric acid, alkan-2-ones (methylketones) and alkan-2-ols.

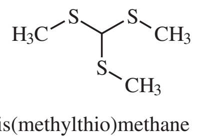


8-185, fatty acid methylthioesters

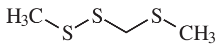
Camembert-type cheeses with a powdery rind of white mold (*Penicillium camemberti*) smell pleasantly of mushrooms, earth and garlic. The characteristic component of the mushroom-like odour is oct-1-en-3-ol, the floral odour components are primarily 2-phenylethanol and 2-phenylethyl acetate, and 1,3-dimethoxybenzene and methyl cinnamate are responsible for the odour resembling nuts. The garlic note of matured cheese is caused by the presence of sulfur compounds, such as 2-bis (methylthio)methane, also known as bis(dimethylsulfanyl)-methane or 2,4-dithiapentane, tris(methylthio) methane, also known as tris(methylsulfanyl)methane or 3-methylthio-2,4-dithiapentane, methyl (methylthio)methyl disulfide, also known as (methyldisulfanyl) methylsulfanylmethane or 2,3,5-trithiahexane, and bis(methylthiomethyl) sulfide (**8-186**).



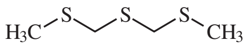
bis(methylthio)methane



tris(methylthio)methane



methyl(methylthiomethyl)disulfide



bis(methylthiomethyl)sulfide

8-186, methylthio and methylthiomethyl compounds

8.2.12.3 Eggs

Fresh eggs contain more than 100 volatile compounds. An important group are the sulfur compounds, mainly dimethylsulfide and dimethyldisulfide, which are characteristic components of egg aroma. Higher levels of these compounds are found in older eggs. Therefore, the long-term storage of eggs produces defects in smell and taste. The degradation products of amino acids, such as indole and lower alkylbenzenes, especially toluene, are also important volatiles. Aldehydes and ketones are present in low amounts (especially acetaldehyde, propionaldehyde, acetone and butanone). A significant proportion of volatiles represent C_7–C_{17} hydrocarbons, with the main hydrocarbon being heptadec-5-ene. Very small amounts of pyrroles and pyrazines are present.

Heat treatment of eggs by cooking and frying creates a variety of other volatile compounds, more from egg yolk than from egg white. The main volatile components are aldehydes, alcohols, free fatty acids, esters and other compounds. Those occurring in the highest quantities are (2*Z*,4*Z*)- and (2*Z*,4*E*)-deca-2,4-dienals, (*Z*)-oct-2-enal, nonanal, hexanal, phenylacetaldehyde and hexyl butyrate. Egg white odour resembles (2*E*,4*Z*,7*Z*)-2,4,7-tridecatrienal, which arises as a product of arachidonic acid autoxidation (see Section 3.8.1.12.1). Its odour threshold was estimated to be 0.07 ng/l air. Sulfane and ammonia are also important protein degradation products.

Some eggs may have an unusual or unacceptable odour or taste, although their appearance is normal. The cause may be age (they are past their 'best'), high storage temperatures, or poor storage conditions, resulting in fishy or other undesirable flavours. The fishy off-flavour, more common in brown-shelled eggs, is caused by the presence of trimethylamine that is produced by microbial decomposition of choline when hens are fed excessive amounts of fishmeals or fish oils and rapeseed and mustard meals. In the case of oilseed meals, trimethylamine arises from sinapine, an ester of choline with sinapic acid. In most breeds of hens, trimethylamine is enzymatically oxidised to odourless trimethylamine oxide, which is excreted from the body.

8.2.12.4 Cereals and cereal products

8.2.12.4.1 Bread and cereal products

Two processes produce aromatic substances from bread. Activities of yeast in the dough give volatiles of breadcrumbs, and baking yields volatiles of bread crust.

The main aroma substances produced by yeast in breadcrumbs are aldehydes, alcohols, acetals and sulfur compounds. The most important components are 2-phenylethanol and 3-methylbutan-1-ol, while the other components present in significant quantities are acetaldehyde, 2-methylpropanal, 3-methylbutanal, ethanol, 2-methylpropan-1-ol, 1,1-diethoxyethane, dimethylsulfide and dimethyldisulfide. In addition, other important volatiles are products of oxidation of fatty acids, especially unsaturated aldehydes (*E*)-non-2-enal and (2*E*,4*E*)-deca-2,4-dienal.

Volatiles of the bread crust arise mainly in the Maillard reaction and caramelisation of sugars. Important precursors of these compounds are the amino acids ornithine, proline, arginine and lysine. Their partners are reducing sugars and their degradation products, for example, methylglyoxal and hydroxyacetone. The basic odorous substances of wheat and rye breads are pyrazines, especially acetylpyrazine and 2-ethyl-3-methylpyrazine. Two key compounds have a major impact on the aroma of bread crust, namely 2-acetyl-1-pyrroline and 6-acetyl-1,2,3,4-tetrahydropyridine, which is in equilibrium with its tautomer 6-acetyl-2,3,4,5-tetrahydropyridine. In wheat bread crust, concentrations of 2-acetyl-1-pyrroline and 6-acetyl-1,2,3,4-tetrahydropyridine were found to be 19–78 and 53 μg/kg, respectively. The precursors of all three compounds are the amino acids proline and ornithine. Ornithine is produced by yeast during fermentation of the dough from glutamic acid, and an alternative route is the formation from proline in a reaction catalysed by ornithine cyclodeamidase. In cases where ornithine is not present, the only precursor is proline. Decarboxylation and oxidation of proline to 1-pyrroline is facilitated by reaction with reactive α-dicarbonyl compounds, such as 1-deoxy-D-*erythro*-hexo-2,3-diulose arising from D-fructose (Figure 4.34), while 1-deoxy-D-*erythro*-hexo-2,3-diulose is via the formation of iminium ion, followed by its decarboxylation and dehydration, transformed into 1,6-dideoxy-D-*glycero*-hexo-2,4,5-triulose (acyclic form of diacetylformoin, Figure 4.40). 1-Pyrroline then condenses with hydrated methylglyoxal to yield 2-(1,2-dioxopropyl)pyrrolidone, which spontaneously oxidises with air oxygen to the corresponding 1-pyrroline, which undergoes an addition of water with the formation of a hydrate and subsequent semibenzilic rearrangement to a β-ketoacid. Its decarboxylation generates 2-acetylpyrrolidine, which is spontaneously oxidised to 2-acetyl-1-pyrroline. The immediate precursor of 2-acetyl-1-pyrroline from ornithine is 4-aminobutanal, which is produced in the Strecker degradation of ornithine, but also as a product of ornithine catabolism by yeast. Ornithine is first decarboxylated to putrescine by ornithine decarboxylase and putrescine transamination yields 4-aminobutanal with catalysis of aminotransferase. A hypothetical mechanism of formation of 2-acetyl-1-pyrroline is outlined in Figure 8.87.

The formation of tetrahydropyridines starts with reaction of 1-pyrroline with hydroxyacetone (acetol, 1-hydroxypropan-2-one), which gives rise to 2-(1-hydroxy-2-oxopropyl)pyrrolidine. The ring enlargement requires opening of the pyrrolidine ring with the formation of 7-aminoheptane-2,3-dione, which finally cyclises to 6-acetyl-1,2,3,4-tetrahydropyridine (Figure 8.88). Hydroxyacetone is produced in the baking process by thermal decomposition of methylglyoxal.

Additional odorous components, such as 3-ethyl-2,5-dimethylpyrazine, biacetyl, 3-methylbutanal, 2-phenylethanal and others are found in dark rye bread. Toasted bread contains the same compounds that carry the aroma of bread crust and crumb. A key component is again 2-acetyl-1-pyrroline, other major components are methional, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol), (*E*)-non-2-enal and biacetyl, the odour of which resembles butter.

Important flavour components of other cereal products are, as in bread, 2-acetyl-1-pyrroline, 6-acetyl-1,2,3,4-tetrahydropyridine, acetylpyrazine and further compounds. Cereal flours and legume

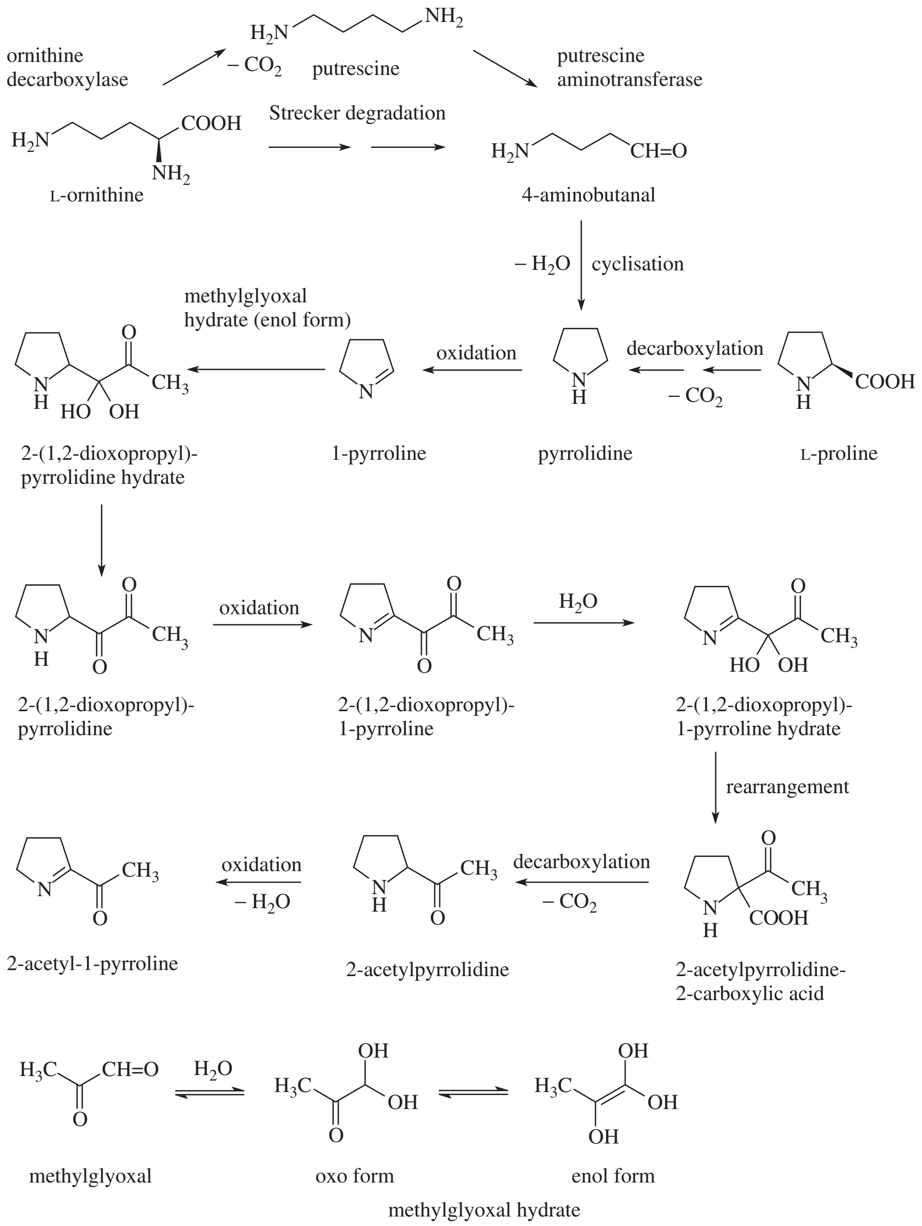


Figure 8.87 Formation of 2-acetyl-1-pyrroline from ornithine and proline.

products, such as soybean products, often assume rancid flavours on prolonged storage. The carriers of this off-flavour are carbonyl compounds (such as hexanal and other aldehydes) produced by oxidation catalysed by lipoxygenases.

8.2.12.4.2 Rice

The aroma of cooked rice (*Oryza sativa*, Poaceae), especially of the aromatic basmati rice, is mainly influenced by the formation of 2-acetyl-1-pyrroline which has the odour of bread crust and popcorn. Its concentration can reach approximately 0.6 mg/kg. Significant compounds are also aldehydes formed by oxidation of fatty acids, especially (2*E*,4*E*)-deca-2,4-dienal, nonanal, hexanal, octanal and (*E*)-non-2-enal, and phenols guaiacol and 4-vinylphenol resulting from the degradation of phenolic acids. Another important compound is 3-hydroxy-4,5-dimethyl-5*H*-furan-2-one (sotolon) with the aroma of protein hydrolysates and 2-aminoacetophenone with a phenolic odour.

8.2.12.5 Fruits

8.2.12.5.1 Apples

The aroma of apples (*Malus pumila*, syn. *M. domestica*, Rosaceae) consists of more than 300 different compounds, of which the most important components are C_5 carboxylic acids, alcohols and esters. Important acids are 2-methylbutyric and 3-methylbutyric (isovaleric) acids, which are found in the fruits in a ratio

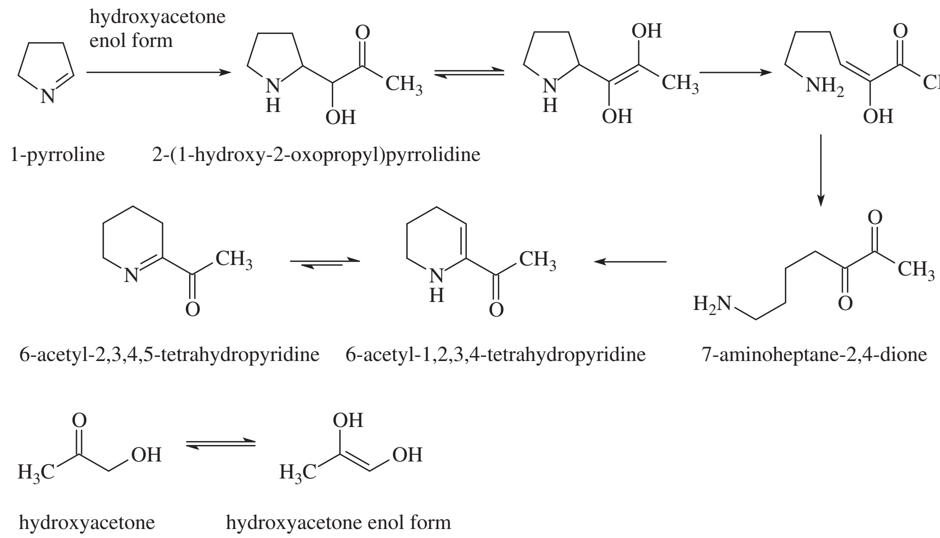


Figure 8.88 Formation of 6-acetyltetrahydropyridines from ornithine and proline.

of about 80 : 20, and in juices in the ratio 99 : 1 due to the activities of microorganisms. In some apple varieties esters predominate, while in others alcohols are in the majority. The key odorous component is ethyl 2-methylbutanoate, but there are other butyanoates and acetates present, such as butyl acetate, 2-methylbutyl acetate and 3-methylbutyl acetate, ethyl butanoate, ethyl 2-methylbutanoate, methyl 2-methylbutanoate, hexyl 2-methylbutanoate and ethylesters of 5-hydroxyoctanoic and 5-hydroxydecanoic acids. Compounds responsible for the green apple aroma are hexanal, (*E*)-hex-2-enal (leaf aldehyde), (*Z*)-hex-3-en-1-ol (leaf alcohol), (*E*)-hex-2-en-1-ol and related esters, such as (*Z*)-hex-3-en-1-yl butyrate. Other alcohols are present, such as butane-1-ol, hexane-1-ol, linalool and 2-phenylethanol. An important flavour-active compound is (*E*)-β-damascenone, resulting from the decomposition of carotenoids, and related compounds, followed by 1-octene-3-one with a mushroom flavour, methional with the aroma of boiled potatoes, and dimethyldisulfide with a sulfurous smell. Cooking of apples causes partial hydrolysis of esters and the formation of lactones from hydroxycarboxylic acids.

8.2.12.5.2 Cherries and plums

A typical component of the flavour in both types of fruits (*Cerasus vulgaris*, syn. *Prunus cerasus* and *Prunus domestica* and other species, Rosaceae) is benzaldehyde. Other important compounds for cherry flavour are linalool, hexanal, (*E*)-hex-2-enal, (2*E*,6*Z*)-nona-2,6-dienal, phenylacetaldehyde and eugenol. During heating of cherry juice and in the preparation of jams and confectionery products, yet more benzaldehyde is produced by hydrolysis of cyanogenic glycosides. Similarly, hydrolysis of linalool glycoside yields linalool. Loss of volatile C_6 aldehydes and nonadienal changes the formerly green aroma to more floral notes. The characteristic aroma components of plums, in addition to benzaldehyde, are linalool, methyl cinnamate, δ-decalactone and aldehydes with green odour. Plum compotes also contain nonanal, benzyl acetate and degradation products of carotenoids.

8.2.12.5.3 Apricots

The aroma of apricots (*Armeniaca vulgaris*, syn. *Prunus armeniaca*, Rosaceae) is composed of a large number of different substances. Important components are monoterpenic hydrocarbons, alcohols and aldehydes (myrcene, limonene, *p*-cymene, terpinolene, α-terpineol, geranial, geraniol and linalool in particular) and aldehydes with green flavour, such as (*Z*)-hex-3-enal and acetaldehyde. Other volatile components include products of oxidation of fatty acids, such as (2*E*,6*Z*)-nona-2,6-dienal, (*Z*)-octa-1,5-dien-3-one, lactones (γ-hexalactone, γ-octalactone, γ-decalactone, γ-dodecalactone, δ-decalactone and δ-dodecalactone), carboxylic acids (especially, 2-methylbutanoic and acetic acids) and degradation products of carotenoids, such as β-ionone.

8.2.12.5.4 Peaches

The basic flavour of peaches (*Persica vulgaris*, syn. *Prunus persica*, Rosaceae) is typified by the presence of γ-lactones (C_6–C_{12}) and δ-lactones (C_{10} and C_{12}). Individual varieties differ mainly in their content of esters and monoterpenoids. As with all stone fruits, an important component is benzaldehyde, while other important compounds include benzyl alcohol, ethyl cinnamate, isopentyl acetate, linalool, α-terpineol, hexanal, (*Z*)-hex-3-enal, (*E*)-hex-2-enal and decomposition products of carotenoids.

8.2.12.5.5 Strawberries

Nowadays, more than 400 compounds forming the large-fruited strawberry (*Fragaria ananassa* and other hybrids, Rosaceae) flavour are known, yet the importance of many of them is not yet fully understood. Besides a number of esters (mainly butanoates) and aldehydes, such as (*Z*)-hex-3-enal, which generally have green and fruity flavours, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol, also known as strawberry or pineapple furanone) and its methyl ether 4-methoxy-2,5-dimethyl-2*H*-furan-3-one

(mesifurane) have special importance for the aroma of ripe fruit. These compounds are present in the order of units of mg/kg. The content of furaneol decreases during ripening and storage of strawberries, but the content of its ether, whose smell is reminiscent sherry type wines, increases. The aroma of strawberry juice reportedly represents a mixture of only furaneol, its methyl ether, (*Z*)-hex-3-enal, methyl and ethyl butanoate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate (ethyl isobutyrate), biacetyl and acetic and butanoic acids. Sulfur volatiles have also been reported in strawberries and some of them may have an impact on strawberry aroma as concentrations of most sulfur volatiles increase with increasing maturity. In addition to hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide, sulfur volatiles of straberries include methyl thioacetate, methyl thiopropionate, methyl thiobutanoate, ethyl thiobutanoate, methyl thiohexanoate, methyl (methylthio)acetate, ethyl (methylthio)acetate, methyl 2-(methylthio)butanoate, methyl 3-(methylthio)propionate, ethyl 3-(methylthio)propionate and methyl thiooctanoate, which can be used to distinguish overripe from full ripe and commercially ripened berries.

8.2.12.5.6 Raspberries

The characteristic flavour component of raspberries (*Rubus idaeus* ssp *vulgatus*, Rosaceae) 4-(4-hydroxyphenyl)butan-2-one is known as raspberry ketone (**8-53**). In addition to this compound, other important compounds include in particular methyl cinnamate, non-1-en-3-one, (*Z*)-hex-3-en-1-ol with a green flavour and degraded carotenoids, such as α-ionone, β-ionone and α-irone.

8.2.12.5.7 Blackcurrants

The characteristic aroma of blackcurrants (varieties of *Ribes nigrum*, Grossulariaceae) is mainly a consequence of aliphatic and alicyclic thiols. The bearer of the characteristic cat odour is 4-methoxy-2-methyl-2-thiol, called blackcurrant mercaptan (**8-124**), which occurs also in olive oil and green tea. Other important components are 4-mercapto-4-methylpentan-2-one called cat ketone (**8-125**) and (1*S*,4*R*)-*p*-menthan-8-thiol-2-one (see Section 8.2.13.1.1). Cat ketone is also found in grapefruits, some hop cultivars, basil and some aromatic wines. The leaves contain about 0.7% essential oil, whose main component is *p*-cymol, the sulfur compounds of which contribute to the strong smell, mainly *p*-menthan-8-thiol-2-one.

8.2.12.5.8 Citrus fruits

The main odour-active components of selected citrus fruits, fruits of plants of the genus *Citrus* of the rut family (Rutaceae), are listed in Table 8.31. The aroma of citrus fruits normally consists of between several tens and several hundreds of compounds. For example, it is estimated that lemon oil contains about 300 different substances. Some of these compounds are of fundamental importance for the character of the typical aroma; others have little or no importance. The main component of all citrus fruits is always (+)-limonene, which determines the basic sensory character of essential oils, but its presence is not necessary. Removal of the limonene while leaving other important components (such as citral) produces a more soluble, stronger and more stable oil, which, however, lacks the characteristic freshness associated with the complete oil. Important components of citrus fruit scents are also saturated straight chain C_8 to C_{12} aldehydes (fatty aldehydes), as well as citral, which is always a mixture of two stereoisomers, known as geranial and neral, and the unsaturated aldehyde (*E*)-dec-2-enal. For the aroma of fresh fruit, aldehydes with green odour are important. Limonene readily undergoes autoxidation and is therefore a source of instability in juices and essential oils obtained from citrus fruits (Figure 8.1).

Oranges

Many compounds are responsible for the smell of oranges. The presence of (+)-valencene distinguishes orange and grapefruit aroma from the aroma of other citrus fruits. Acetaldehyde and (*Z*)-hex-3-enal are the main contributors to the smell of fresh fruit. Some aldehydes also influence the aroma of oranges, for example octanal, nonanal and decanal, and deca-2,4-dienals are important components in Spanish oranges. β-Sinensal has a pleasant orange smell (α-sinensal is typical for the aroma of mandarin); other important compounds are citral (mixture of isomers) and vanillin and some ketones, such as pent-1-en-3-one and β-ionone. The aroma of fresh oranges can be substituted by about 15 components that also include some ethyl esters, such as ethyl propionate, ethyl butanoate, ethyl 2-methylpropanoate, (*S*)-ethyl 2-methylbutanoate, ethyl 3-hydroxyhexanoate, neryl acetate and wine lactone. Important alcohols include 3-methylbutan-1-ol, (+)-α-terpineol, (−)-terpinen-4-ol and (+)-linalool. Other significant components are, furthermore, some hydrocarbons, especially β-caryophyllene with spice-like odour, α-pinene and myrcene.

The composition of volatiles of juices and beverages prepared from the oils extracted from citrus fruit peels is slightly different. For example, juices contain ethyl vinyl ketone, (*E*)-pent-2-enal and ethyl butanoate as major aromatic substances.

The main products of limonene oxidation, carvone and carveol, cause a terpenic off-flavour in essential oils and juices. Valencene oxidation products, such as (+)-nootkatone, cause a grapefruit-like off-flavour in orange juices. Large amounts of alcohol (+)-α-terpineol, which arises, for example, during storing of juices by acid catalysed hydration or microbial transformation of limonene, is also perceived as an off-flavour.

Bergamot oranges

The essential oil of bergamot oranges is rich in (+)-limonene, (+)-linalool and (+)-linalyl acetate. The dominant sesquiterpenes are (*E*)-α-bergamotene (0.2–0.4%), and other major sesquiterpenes are β-caryophyllene, germacrene D, α-humulene, β-farnesene and β-bisabolene. The typical aroma of bergamot oranges is attributed to oxygen compounds, such as (−)-guaienol, (+)-spathulenol, nerolidol, farnesol, α-bisabolol and β-bisabolol. Other important trace compounds are aldehydes β-sinensal, lanceal and bergamotenal. Further compounds that make a contribution are sesquiterpenic ketones (+)-nootkatone, (+)-8,9-didehydronootkatone, camphorenone and certain other substances.

Table 8.31 Basic composition of citrus essential oils (in %).

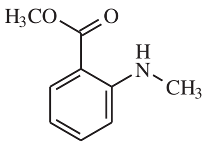
Compound	Orange (*C. aurantium var. dulcis*)	Bergamot orange (*C. a. var. bergamia*)	Mandarin orange (*C. reticulata*)	Lemon (*C. limonum*)	Grapefruit (*C. paradisi*)
Hydrocarbons					
α-Bergamotene	0.06	0.3	–	0.4	–
β-Bisabolene	–	0.6	–	0.2–0.8	–
Camphene	–	–	0.02	0.2–0.5	0.01–0.4
Caryophyllene	0.1	0.2	0.03	0.3	0.02–0.1
p-Cymene	0.2	0.5–3.5	0.2–8	0.6–1	0.4
Farnesenes	0.07	0.04	0.1	–	0.1
Limonene	88–97	26	80–94	60–80	86–95
Myrcene	1–2	0.6–1.1	2–7	1–12	2–3.7
α-Phelandrene	–	–	0.05–0.3	0.2	–
β-Phelandrene	0.1	0.1	0.5	0.8	–
α-Pinene	0.2–0.6	1–2.2	1–2.5	1.5–5	0.4
β-Pinene	–	4–10	1–2	6–14	0.05
Sabinene	0.2–0.6	1	1–2	0.8–1.9	1.1
α-Terpinene	–	–	0.1–0.4	0.7	–
β-Terpinene	–	3.2	–	–	–
γ-Terpinene	0.1	5–11	3–17	6–12	0.1–0.8
Terpinolene	0.1	0.3–1	–	0.6–0.9	–
Valencene	0.2	–	–	–	0.4
Alcohols					
Citronellol	–	–	0.02	0.5	–
Fenchol	–	0.01			–
Geraniol	–	0.6	0.04	0.1	–
Linalool	0.55	16	1–6	0.2	0.4
Nerol	–	0.08	0.05	0.1	–
Octan-1-ol	–	0.02	0.1	–	0.8
Terpinen-1-ol	–	–	0.2	–	–
α-Terpineol	0.1–0.5	0.3	0.05–1	0.3	0.2
β-Terpineol	–	–	0.4	–	–
Terpinen-4-ol	0.06–0.2	0.06	0.06–0.3	0.01–0.4	0.08
Aldehydes					
Citral	0.1–0.2	0.6	0.2	2–13.2	–
Citronellal	0.1	0.02	0.15	0.03–0.2	0.1
Decanal	0.1–0.7	–	0.2–0.9	0.15	0.3–0.6
Dodecanal	0.05–0.2	–	0.02–0.15	0.1	0.15
Nonanal	0.06–0.2	0.08	0.03	0.1–0.3	0.04–0.1
Octanal	0.2–2.8	0.07	0.3	0.15	0.3–0.8

Table 8.31 (*continued*)

Compound	Orange (*C. aurantium var. dulcis*)	Bergamot orange (*C. a. var. bergamia*)	Mandarin orange (*C. reticulata*)	Lemon (*C. limonum*)	Grapefruit (*C. paradisi*)
Perillaldehyde	0.02	-	0.02–0.1	-	0.2
α-Sinensal	0.03	-	0.2	-	-
β-Sinensal	0.1	-	0.2	-	-
Ketones					
Carvone	0.1	0.09	0.005–0.03	0.04	0.02
Nootkatone	0.01	-	0.01	0.06	0.5–1.8
Esters					
Citronellyl acetate	-	-	0.1	0.2	0.04
Geranyl acetate	-	-	0.1	0.1–1	0.2
Neryl acetate	0.1	-	0.1	0.7	0.2
1-Octyl acetate	0.1	-	0.3	0.04	0.1

Mandarin oranges

Unlike other citrus essential oils, tangerine essential oil contains about 0.85% of *N*-methyl methylanthranilic acid (**8-187**), which is the key component. Other important substances are terpenic hydrocarbons, such as γ-terpinene and β-pinene, terpenic aldehyde α-sinensal and other terpenoids.



8-187, methyl *N*-methylanthranilate

Grapefruits

The key components of fresh grapefruit juices with a typical grapefruit odour are both isomers of *p*-mentha-1-en-8-thiol (**8-188**). The (+)-(*R*)-enantiomer is present in minute concentrations (less than 1 μg/kg), but has a very low odour threshold concentration. The (−)-(*S*)-*p*-mentha-1-ene-8-thiol has a weak and non-specific smell. Of the other sulfur compounds, 4-mercapto-4-methylpentan-2-one (**8-125**) is significant, and also occurs in blackcurrants, some hop cultivars, aromatic wines and basil. A relatively high content of sesquiterpenoids is also typical. The smell and bitter taste of grapefruits arise from (+)-nootkatone and (+)-8,9-didehydronootkatone. Important odour-active compounds are numerous cyclic ethers, which are likewise found in other essential oils. For example, the essential oil contains about 13% of linalool oxides that arise from linalool via 5,6-epoxide, and another important epoxide is (*E*)-4,5-epoxydec-2-enal. The fresh odour of juices is mainly influenced by aliphatic aldehydes, such as acetaldehyde, (*Z*)-hex-3-enal and decanal, as well as by some esters, such as 2-methylpropanoate, (*S*)-2-methylbutanoate and wine lactone.

(*R*)-*p*-menth-1-ene-8-thiol (*S*)-*p*-menth-1-ene-8-thiol

8-188, *p*-menth-1-ene-8-thiol enantiomers

Lemons

Principal carriers of the smell of lemons are β-pinene, (−)-terpinen-4-ol and citronellol, whose smell is reminiscent of lemon peel, while α-bergamotene contributes significantly to the basic lemon odour. A high content of citral (isomeric aldehydes geranial and neral) and the presence of some alkanals, esters and alcohols, especially geranyl acetate, neryl acetate and α-bisabolol are very important.

The off-flavour of lemon juices may be caused by degradation products of citral (*p*-cymene and α,*p*-dimethylstyrene) that result from reactions catalysed by acids.

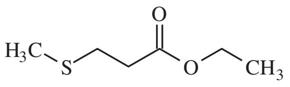
8.2.12.5.9 Bananas

The characteristic aroma components of bananas (*Musa* x *paradisiaca*, Musaceae) are esters. Important components are largely acetic acid esters, and the most significant compound is isopentyl acetate. The typical banana odour comes from esters of pentan-1-ol with acetic, propionic and butyric acids, while esters of butanols and hexanols with acetic and butyric acids generally show a fruity aroma. Other compounds also contribute to the full fine aroma,

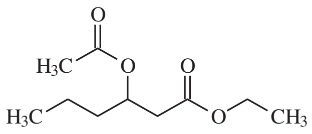
such as eugenol and its derivatives (methyleugenol and elemicin) and other compounds.

8.2.12.5.10 Pineapple

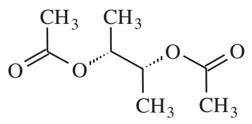
Pineapple (*Ananas comosus*, Bromeliaceae) aroma consists of about 200 alcohols, esters, lactones, aldehydes, ketones, monoterpenes, sesquiterpenes and other volatiles. About 80% of the total volatile substances are esters. The main components in the green fruit are ethyl acetate, ethyl 3-(methylthio)propionate (**8-189**) with a distinctive pineapple aroma and ethyl 3-(acetoxy)hexanoate (**8-190**). The ripe fruit contains, as the main esters, ethyl acetate, (2*R*,3*R*)-butane-2,3-diol diacetate (**8-191**) and ketone 3-hydroxybutan-2-one. An important compound for the typical character of pineapple aroma, as in strawberry aroma, is 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (furaneol), present as a glycoside, and 2,5-dimethyl-4-methoxy-2*H*-furan-3-one.



8-189, ethyl 3-(methylthio)propanoate



8-190, ethyl 3-(acetoxy)hexanoate



8-191, (2*R*,3*R*)-butan-2,3-diol diacetate

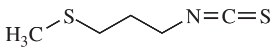
8.2.12.6 Vegetables

8.2.12.6.1 Cabbage, kale and kohlrabi

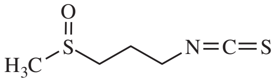
The aroma of cabbage (*Brassica oleracea* var. *capitata*), kale (*B. o.* var. *sabauda*), kohlrabi (*B. o* var. gongylodes) and other vegetables of the genus *Brassica* (Brassicaceae) is characterised by the presence of isothiocyanates, which result from the enzymatic and non-enzymatic decomposition of glucosinolates. For example, cooking cabbage produces, as the main products, allyl isothiocyanate (prop-2-en-1-yl isothiocyanate) arising from sinigrin, but-3-en-1-yl isothiocyanate (from gluconapin) and 2-phenylethyl isothiokyanate (from gluconasturtiin). Especially important is the latter compound, as it has a low odour threshold concentration. The resulting aroma, in addition to isothiocyanates, is also influenced by a large number of other sulfur compounds, such as dimethylsulfide and dimethyltrisulfide that are formed by decomposition of *S*-methylcysteine sulfoxide, cysteine and methionine (via methional). Nitriles produced as byproducts of degradation of glucosinolates also have some relevance, and to some extent recall the aroma of garlic. The main component is allyl cyanide (but-3-ene nitrile).

8.2.12.6.2 Cauliflower and broccoli

The components of cooked cauliflower (*Brassica oleracea* var. botrytis) and broccoli (*B. o* var. *italica*) aroma are similar to the aroma components of cabbage. Another important compound is the aldehyde nonanal. The typical odour of cauliflower comes from 3-(methylthio)propyl isothiocyanate (**8-192**), which is produced from glucosinolate glucoibervirin. The typical odour of broccoli is also due to the presence of 3-(methylsulfinyl)propyl isothiocyanate (**8-193**), which develops from glucosinolate glucoiberin.



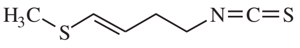
8-192, 3-(methylthio)propyl isothiocyanate



8-193, 3-(methylsulfinyl)propyl isothiocyanate

8.2.12.6.3 Radish and horseradish

The aroma of radishes and horseradish of the Brassicaceae family, as well as the aroma of *Brassica* vegetables, is influenced by the presence of sulfur compounds resulting from the decomposition of glucosinolates. The main component of numerous varieties of radishes (*Raphanus sativus*) is (*E*)-4-(methylthio)but-3-ene-1-yl isothiocyanate (**8-194**), which is produced from glucosinolate glucoraphasatin and is responsible for pungent taste of many radishes. The odour and pungent taste of horseradish (*Armoracia rusticana*) is caused by the presence of allyl isothiocyanate, which is a degradation product of sinigrin.



8-194, (*E*)-4-(methylthio)but-3-ene-1-yl isothiocyanate

8.2.12.6.4 Carrots

The aroma of carrots root (*Daucus carota*, Apiaceae) is very complex. It consists of various aldehydes, ketones, mono- and sesquiterpenic hydrocarbons and other compounds. The important hydrocarbons are myrcene, sabinene, terpinolene, β-caryophyllene, γ-bisabolene and α-pinene, which are present in the largest quantities. The significant carbonyl compounds are acetaldehyde and (2*E*,6*Z*)-nona-2,6-dienal. One of the typical aromatic substances determining the basic odour of carrots is 2-*sec*-butyl-3-methoxypyrazine. During the cooking of carrots, the contents of methanal, ethanal, propanal, octanal, (*Z*)-dec-2-enal and some sulfur compounds, such as dimethylsulfide and ethanethiol, increase. At the same time, the contents of monoterpenes and β-caryophyllene decrease.

8.2.12.6.5 Parsley

The cause of the typical smell of fresh parsley (*Petroselinum hortense*) is *p*-mentha-1,3,8-triene (Figure 8.9). As in other vegetables of the Apiaceae family, important substances of root parsley and leaf parsley are phthalides. The main phthalides are sedanenolide, (*E*)-ligustilide and butylphthalide, while (*Z*)- and (*E*)-butylidene-phthalide, (*Z*)-ligustilide, (*Z*)-sedanolide and 3-butyl-5,6-dihydrophthalide are present in smaller amounts. Other important components of leaf parsley are linalool, β-citronellol, methyl 2-methylbutanoate, oct-1-en-3-one, (*Z*)-octa-1,5-dien-3-one, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, (*Z*)-hex-3-enal, (*E*)-dec-6-enal, (2*E*,4*E*)-deca-2,4-dienal and β-ionone resulting from the degradation of carotenoids. Other components are the monoterpenes myrcene, α-pinene, β-pinene, α-thujene, camphene, sabinene, car-3-ene (Δ^3-carene), α- and β-phellandrene, (*S*)-limonene, γ-terpinene, *p*-cymene and terpinolene. *p*-Mentha-1,3,8-triene is the main aroma constituent of parsley leaf oil.

8.2.12.6.6 Celery and celeriac

The typical components of the aroma of celery (*Apium graveolens* var. *rapaceum*, Apiaceae) and celeriac (*A. g.* var. *dulce*) are numerous dihydrophthalides, tetrahydrophthalides, hexahydrophthalides and phthalides. The main odour-active compound is 3-butyl-4,5-dihydrophthalide (sedanenolide) and 3-butyl-3,4,5,6-tetrahydrophthalide (sedanolide, neoknidilide), 3-butylphthalide and 3-butylidene-4,5-dihydrophthalide, known as (*Z*)-ligustilide, are likewise present in significant quantities. Also present in smaller amounts are other dihydrophthalides, tetrahydrophthalides and hexahydrophthalides.

8.2.12.6.7 Beetroot

The odour of raw and cooked beetroot, also known as table beet, red beet or simply as beet (*Beta vulgaris* var. *conditiva*, Amaranthaceae, formerly Chenopodiaceae), is strongly influenced by the presence of tertiary sesquiterpenic alcohol (−)-geosmin (**8-23**), which is responsible the earthy smell of this vegetable. It is produced in the soil by some microorganisms. Other important compounds include pyridine, 4-methylpyridine, 2-*sec*-butyl-3-methoxypyrazine, some carbonyl compounds, such as 3-methylbutanal, biacetyl, hexanal, furan-2-carbaldehyde, benzaldehyde and phenylacetaldehyde, and alcohols, for example 2-methylpropan-1-ol and 3-methylbutan-1-ol.

8.2.12.6.8 Tomatoes

The aroma of tomatoes (*Solanum lycopersicum*, syn. *Lycopersicum esculentum*, Solanaceae) represents about 400 different compounds. Important components of the aroma of fresh ripe tomatoes are 2-isobutylthiazole, which is also the bearer of the typical aroma of the whole plant. The precursors of 2-isobutylthiazole are 3-methylbutanal, which arise by deamination and decarboxylation of leucine, and cysteamine (2-mercaptoethylamine) are formed by decarboxylation of cysteine (Figure 8.85). Important components of tomato aroma are also C_6 aldehydes (*E*)-hex-2-enal, hexanal and (*Z*)-hex-3-enal that are products of enzymatic oxidation of fatty acids, 3-methylbutanal, 2-methylbutanal and the corresponding alcohols are formed from amino acids. Oxidation products of carotenoids also play a key role, such as unsaturated methylketones (*E*)-6-methylhept-5-en-2-one and (*E*)-6-methylhepta-3,5-dien-2-one and other products, such as β-ionone, β-cyclocitral, 5,6-epoxy-β-ionone and (*E*)-β-damascenone. A significant odorous substance is 2,5-dimethyl-4-hydroxy-2*H*-furan-3-one (furaneol), which occurs in the intact form of β-D-glucoside.

The aroma of thermally processed tomatoes, such as tomato paste, is mainly determined by the presence of acetic and isovaleric (3-methylbutanoic) acids, 3-methylbutanal, methional, eugenol, 4-vinylguaiacol, dimethylsulfide, β-ionone and (*E*)-β-damascenone. There are other less active components, such as linalool, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) and its lower homologue 4-hydroxy-5-methyl-2*H*-furan-3-one (norfuraneol). The aroma of tomato concentrates is influenced by the presence of some Maillard reaction products, such as 2-acetylpyrrole, 2-formylpyrrole and some pyrazines.

8.2.12.6.9 Bell peppers

Bell pepper (*Capsicum annuum*, Solanaceae), also known as sweet pepper, pepper or capsicum, and chilli pepper (*C. frutescens*), contains as the key compound 2-isobutyl-3-methoxypyrazine (**8-175**), which carries the typical sharp spice-like odour of fresh vegetables and has an extremely potent odour. Other major components identified include terpenic hydrocarbons and alcohols, such as (*E*)-β-ocimene, limonene and linalool, methyl salicylate, aldehydes (2*E*,6*Z*)-nona-2,4-dienal (as in cucumbers) and (2*E*,4*E*)-deca-2,4-dienal and (*Z*)-hex-3-en-1-ol produced from unsaturated fatty acids.

8.2.12.6.10 Cucumbers

The key odour-active components of fresh cucumbers (*Cucumis sativus*, Cucurbitaceae) are aldehydes generated by enzymatic oxidation of unsaturated fatty acids. The most important compounds are (3*Z*,6*Z*)-nona-3,6-dienal, (2*E*,6*Z*)-nona-2,6-dienal and (*Z*)-non-3-enal, which recall the smell of fresh cucumbers. Additional important components are some other aldehydes, such as (*Z*)-hex-3-enal, (*E*)-hex-2-enal, (*E*)-non-2-enal, nonanal, (*Z*)-hex-6-enal, certain alcohols and 2-alkyl-3-methoxypyrazines.

The odour of fermented cucumbers and cucumbers pickled in sour brine is almost exclusively influenced by the odours of the fermentation products used, such as vinegar and spices.

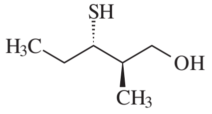
8.2.12.6.11 Garlic

The characteristic aroma of fresh garlic (*Allium sativum*, Amaryllidaceae) is almost exclusively represented by diallyl thiosulfinate (allicin) and to a lesser extent by allylmethyl thiosulfinate. The aroma of heat-treated garlic is associated with degradation products of thiosulfinates, especially diallyl sulfide, diallyl disulfide, diallyl

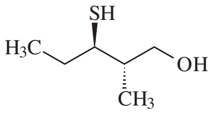
thiosulfonate, allylthiol, allylmethyl disulfide and vinyldithiines. At higher temperatures (during frying or baking) a number of dithiolanes and trithiolanes, dithianes, trithiepanes and tetrathiepanes are produced.

8.2.12.6.12 Onions, leeks and chives

The most important aromatic compounds of freshly sliced onions (*Allium cepa*, Amaryllidaceae), leeks (*A. ampeloprasum*) and chives (*A. schoenoprasum*) are dialk(en)yl thiosulfinates (prop-1-en-1-yl-, methyl and propyl derivatives) that give rise to a diverse mixture of odour-active degradation products. The tear factor of onion is (*Z*)-thiopropanal-*S*-oxide (Figure 8.63). The odorous constituent of raw and heat-treated onion is the intensively smelling 3-mercapto-2-methylpentan-1-ol (Figure 8.86). Its content in freshly cut raw onion ranges from 8 to 32 μg/kg, while in raw stored and then cooked onion its content rises to 34–246 μg/kg. The odour perception thresholds of (2*R*,3*S*)- (**8-195**) and (2*S*,3*R*)-isomers (**8-196**) are 0.04 and 0.03 μg/l in water, while the odour thresholds of the other two isomers are more than 100 times higher. Spontaneous degradation of dialk(en)yl thiosulfinates yields the corresponding disulfides and trisulfides that, together with 2-methylbut-2-enal and 2-methylpent-2-enal, represent the main aromatic components of cooked onions. The characteristic aroma components of fried onions are dimethylthiophenes, especially 2,4-dimethylthiophene and 3,4-dimethylthiophene (Figure 8.74).



8-195, (2*R*,3*S*)-3-mercapto-2-methylpentan-1-ol



8-196, (2*S*,3*R*)-3-mercapto-2-methylpentan-1-ol

8.2.12.6.13 Peas

Essentially important for the odour of green peas (*Pisum sativum*, Viciaceae) are aldehydes and some pyrazines. The typical smell is of 2-isopropyl-3-methoxypyrazine, an important component is also 2-*sec*-butyl-3-methoxypyrazine, which resembles the odour of bell peppers.

8.2.12.6.14 Asparagus

The characteristic aroma component of asparagus (*Asparagus officinalis*, Asparagaceae) is 3*H*-1,2-dithiol, which is produced during cooking from asparagusic (1,2-dithiolane-4-carboxylic) acid (Figure 8.89), which forms a redox system together with dihydroasparagusic acid. Asparagusic acid is present as free acid and as methyl and ethyl esters together with the methyl esters of

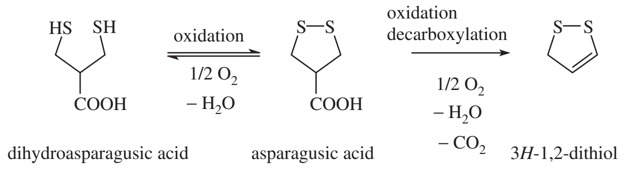
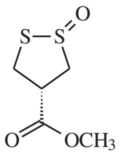


Figure 8.89 Decomposition of asparagusic acid during boiling of asparagus.

the epimeric sulfoxides of asparagusic acid, in which the *S*-oxide takes up either a *syn* or *anti* configuration relative to the methyl ester (**8-197**). A minor constituent of asparagus is 1,2,3-trithiane-4-carboxylic acid, which is a contact allergen from asparagus. Other aromatic compounds of cooked asparagus are dimethyl sulfide, various thiophenes, thiazoles (e.g. 2-acetylthiazole), pyrroles, pyrazines, aldehydes, ketones and phenols (e.g. vanillin).

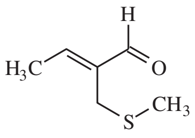


8-197, asparagusic acid *anti*-*S*-oxide methyl ester

8.2.12.7 Potatoes

2-Isopropyl-3-methoxypyrazine and 2,5-dimethylpyrazine, with their typical earthy flavour, have great importance for the aroma of raw potatoes (*Solanum tuberosum*, Solanaceae). In addition to these compounds, raw potatoes contain a range of carbonyl compounds and alcohols. A very important component of the aroma of cooked potatoes is methional.

The aroma of potato chips (French fries) is a mixture of more than 500 compounds. The important components are 2,3-diethyl-5-methylpyrazine, 2-ethenyl-3-ethyl-5-methylpyrazine, (2*E*,4*Z*)-deca-2,4-dienal, (*E*)-4,5-epoxydec-2-enal (8-183), 1-octen-3-one, (*Z*)- and (*E*)-non-2-enal, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, biacetyl and 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol). Another principal component is (*Z*)-2-(methylthiomethyl)but-2-enal (also known as 2-ethylidene methional, **8-198**), which arises in the reaction of methional with acetaldehyde. Both isomers of 2-(methylthiomethyl)but-2-enal are used as flavour enhancers. Less significant compounds are methanethiol and methional.



8-198, (*Z*)-2-(methylthiomethyl)but-2-enal

8.2.12.8 Alcoholic beverages

The characteristic aroma of beer, wine, spirits and other alcoholic beverages is formed by a large number of volatile compounds that are formed due to activities of yeasts or come from the raw materials used. In addition to fermentable sugars, higher fatty acids, organic nitrogen and sulfur compounds and many other substances pass into yeast cells from the media, and these substances participate in biochemical reactions in which aromatic components are formed as byproducts. Their number depends on the conditions during fermentation, and also, in spirits, on the method of distillation. A number of components arise by chemical reactions during ripening and storage of beverages.

8.2.12.8.1 Hops and beer

Worldwide, several hundred brands of beer are produced. Types of beer vary, being based on the basic classification into ales and lagers, which roughly corresponds to the division between bottom-fermented and top-fermented beers. Beers contain about 1000 different compounds, many of which can affect the organoleptic properties, which depend on the raw materials used and their quantity, brewing technology, the yeast used, possible contamination by other microorganisms and also on other factors. According to the current taxonomy, the yeasts used to produce alcoholic beverages are classified under one common species named *Saccharomyces cerevisiae*. Previously used names for bottom-fermented yeast were *S. carlsbergensis* and later *S. uvarum*, and *S. cerevisiae* was the name for top-fermenting yeast. The bottom-fermented yeast was also classified as a subspecies of *S. cerevisiae* yeast.

The primary aromatic substances in beer are derived from raw materials (barley or hops) that confer the beer's typical odour and taste. Bitter acids of hops have a bitter taste (see Section 8.3.5.1.3), but hop cones also contain 0.3–1% m/m of terpenoids (60–80% of hop essential oil), which have a considerable influence on the smell of beer. The main components of aromatic hop oils are sesquiterpenic hydrocarbons in which α-humulene, β-caryophyllene and farnesene dominate. The major monoterpenic hydrocarbon is myrcene. For example, the essential oil content of fine aromatic varieties, such as Saaz, is 0.8% m/m, of which 23% is myrcene, 20.5% α-humulene, 14% farnesene 6% and β-caryophyllene. Significant components of the hop aroma in beer are mainly isomeric terpenoid monoepoxides resulting from autoxidation and diepoxides of α-humulene and β-caryophyllene, but also other terpenoids. Important components of hops odour are also various alcohols (such as geraniol and linalool), esters (ethyl 2-methylpropanoate, methyl 2-methylbutanoate, propyl 2-methylbutanoate and esters of terpenic alcohols, such as geranyl isobutanoate), hydrocarbons, aldehydes and ketones formed by oxidation of fatty acids, such as (3*E*,5*Z*)-undeca-1,3,5-triene, (*Z*)-hex-3-enal, nonanal, (*Z*)-octa-1,5-dien-3-one, their epoxides, such as (*E*)-4,5-epoxydec-2-enal and sulfur compounds. Other important components of hops are so-called polyphenols (condensed tannins) that influence the beer's taste and have antioxidant effects. Less important compounds are waxes and other lipids. Hop products, such as powder, pellets and extracts (by extraction with carbon dioxide or organic solvents, such as dichloromethane, hexane or ethanol), are thus derived from hops.

Other aromatic substances are formed by enzymatic and chemical reactions during malting, and many compounds arise during the technological processes, such as mashing and fermentation. Fermentation changes the smell and taste of a young beer by reactions related to the metabolism of yeast. Top-fermented beers are sometimes very aromatic. Some beers, especially wheat beers, may contain a higher amount of 4-vinylguaiacol, which grants them a typical flavour. Other flavours can come from the addition of various spices, such as coriander, which is added to some Belgian beers.

Generally, some of the odour-active components of beer are considered desirable or desirable in small quantities, while others are indifferent or undesirable. For example, for the Pilsner type of beer, important odourous components include some alcohols (ethanol and linalool), aldehydes (acetaldehyde), acids (2- and 3-methylbutanoic acids), ethyl esters (butanoate, 2-methylpropanoate, 4-methylpentanoate and caprylate), sugar degradation products, such as of 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) and degradation products of carotenoids, such as (*E*)-β-damascenone. Interestingly, the feeling of an empty taste is associated with lower ethanol content. The bearers of caramel aroma in dark beers are maltol and isomaltol, which can come from caramel malts used in the production of dark beers, or from caramel used for colouring. Various esters of organic acids, such as 3-methylbutyl acetate and ethyl esters of fatty acids, have a flowery and ester aroma. An important descriptor of the taste of beer is called fullness, which may be caused by increased extract in unfermented beer, higher protein and carbohydrate content, and by the composition of the brewing water, which may for example have increased chloride content. Carbon dioxide content also significantly affects the physiological and sensory perception and beer foaming.

A range of different reasons may lead to the formation of undesirable sensory active substances, such as microbial contamination, pasteurisation and long-term storage. Various secondary substances, formed during the fermentation, pasteurisation and during long-term storage, may affect the smell/taste profile of beer – usually negatively. The carriers of sulfur odour are sulfane and various thiols, dimethyl sulfide and dimethyltrisulfide (arising either from hop *S*-methylcysteine sulfoxide or methional). Their smell resembles cooked vegetables, biacetyl smell resembles butter, acetic acid has a smell resembling vinegar, volatile phenols cause a phenolic aroma, and some lactones (especially δ-decalactone) give rise to a fruity odour. The development of a solvent-like stale flavour is associated with the formation of furfuryl ethyl ether, which is formed in an acid-catalysed substitution reaction of ethanol and furfuryl alcohol formed in the Maillard reaction. Various physical factors accelerate the aging of beer, particularly temperature and light radiation. During transport and storage, beer may be exposed to the effects of visible light (in the presence of photosensitisers, such as riboflavin), leading to an old, cardboard-like flavour caused by the formation of alkenals represented by 3-methylbut-2-en-1-thiol. The oxidation of unsaturated fatty acids to (*E*)-non-2-enal (and other products) leads to an off-flavour known as light-struck (cardboard) flavour (see Section 8.2.9.1.2) in

aged beers when present at levels as low as 0.035 μg/l). Concentrations of (*E*)-non-2-enal ranging from 0.2 to 0.5 g/l are usually found after 3–5 months at 20 °C, but then decrease below the threshold concentration after one year of aging, which is probably due to hydration to 3-hydroxynonanal or oxidation to non-2-enoic acid.

8.2.12.8.2 Grapes and wine

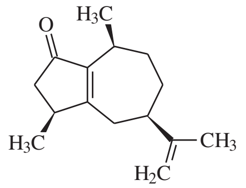
The organoleptic properties and quality of wine are influenced by many different factors. The quality of odour and taste depends on the balance of individual constituents. Specific wines are different depending on the vine variety, the ripeness of the grapes, the eventual attack by microorganisms (especially by *Botrytis cinerea* fungi), conditions during must fermentation (a dominant species of microorganisms are yeast *Saccharomyces cerevisiae*), such as temperature, and other factors.

Compared with other beverages prepared by fermentation, wines are very acidic (their pH value is within 2.8 to 3.8, and tartaric acid is a major carrier of sour taste) and they have a relatively high ethanol content. Ethanol has a significant influence on the nature of the taste. Its content is related to sugar content in the must, and therefore it is related to the degree of grape ripeness and the extent of the fermentation. For the red wine taste, tannins, condensation products of catechins and other flavonoid molecules are important compounds. Ethanol corrects their bitter and astringent taste.

It is estimated that wines contain 400–600 flavour-active compounds in a total amount of 0.8–1.2 g/l. In wine terminology, 'aroma' is the term used for the smell of young wines. The transformation of aroma during aging by chemical reactions leads to a wine's bouquet. Wine flavour is classified into primary – the bearer of which are substances present in grapes and musts – and secondary, the bearer of which are substances produced during fermentation.

According to the grape varieties, wines can have neutral (such as Müller Thurgau) and strong aroma (such as Muscat, Sauvignon, Gewürztraminer and Scheurebe). The primary aroma of muscat grapes is determined by the presence of ten terpenic alcohols, especially linalool (the content is about 0.4 mg/l, odour threshold concentrations in wine is 0.1 mg/l), geraniol (0.3 mg/l, odour threshold concentration of 0.1 mg/l), nerol (0.1 mg/l, odour threshold concentration of 0.4 mg/l), α-terpineol (0.1 mg/l, odour threshold concentration of 0.5 mg/l) and linalooloxides (0.1 mg/l, odour threshold concentrations of 3–7 mg/l). The characteristic impact components of Gewürztraminer and Scheurebe wines are *cis*-rose oxide and 4-mercapto-4-methylpentan-2-one (**8-125**). Some of the above terpenes are also involved in the odour of other grape varieties (such as Riesling), where their concentration is lower and only reaches 0.1–0.3 mg/l. In varieties lacking the characteristic aroma, these terpenoids are present only in traces, or not at all.

The peppery character of grapes and wines is attributed to the presence of the sesquiterpenoid (–)-rotundone, (3*S*, 5*R*,8*S*)-5-(isoprop-2-en-1-yl)-3,8-dimethyl-3,4,5,6,7,8-hexahydro-2*H*-azulen-1-one (**8-199**). Rotundone has a very distinctive aroma and low sensory threshold concentrations (16 ng/l in red wine and 8 ng/l in water). This guaiane-like compound was first found in Syrah (Siraz), Mourvèdre and Durif wines produced in Australia, at concentrations of up to 145 ng/l and later in higher amounts (up to 561 ng/l) in Schioppettino and Vespolina red wines and in Gruener Veltliner white wines produced in Europe. Rotundone was also found in much higher amounts in some common herbs and spices, such as black pepper, marjoram, oregano, rosemary, basil and thyme. In black and white peppercorns it is present at about 10 000 times higher levels than found in very peppery wine.



8-199, (–)-rotundone

The secondary aromatic compounds are mainly higher alcohols (Table 8.5) and esters (Table 8.25). Higher alcohols are present in somewhat larger quantities than those that correspond to their odour thresholds. At low concentrations they have a positive effect on the wine aroma, but in concentrations higher than about 400 mg/l they have a negative effect on wine aroma. An important role is played by ethyl acetate. In sub-threshold concentrations of 50–80 mg/l (the odour threshold concentration in wine is 160 mg/l), together with other substances, ethyl acetate is a desired aroma component, but in concentrations higher than 160 mg/l it has a harsh taste and smell. For the particular aroma of white wines, ethyl esters of fatty acids are also important (such as caproic, caprylic, capric and lauric acids), and occur in relatively low concentrations (5–10 mg/l), which are nevertheless about ten times higher than the threshold concentrations. The smell of ethyl caproate is fruity, but the smell of ethyl laurate is soap-like.

Thiol-containing compounds released from non-volatile precursors during alcoholic fermentation, in particular enantiomers of 4-mercapto-4-methylpentan-2-one, 4-mercapto-4-methylpentan-2-ol and 3-mercaptohexan-1-ol (**8-125**, **8-126**), are responsible for citrus and grapefruit scents of Sauvignon Blanc white wines and some other grape varieties (e.g. Gewurztraminer, Riesling, Muscat, Sylvaner, Cabernet Sauvignon and Merlot). For example, the aroma of 3-mercaptohexan-1-ol (0.03–13 μg/l) resembles grapefruits and passionfruit. Various other thiols have likewise been identified in wines, such as 2-furanmethanethiol and benzylmercaptan.

Certain heterocyclic compounds are also important aromatic substances in wines, such as pyrazines in Cabernet Sauvignon and Sauvignon Blanc wines (see Section 8.2.11.1.7) and both enantiomers of 3-hydroxy-4,5-dimethyl-5*H*-furan-2-one (sotolon), which occur in white wines, sherries and are a key component of the typical aroma of aged Port wines. The precise chemical reactions leading to the formation of bouquet substances are not yet widely known. There are two types of reactions that produce bouquet constituents: oxidation, which is characterised by the presence of aldehydes and acetals (e.g. in Madeira-type wines) and reduction (such as in quality table wines after a period of bottle maturation; the flavour of low-quality wines does not improve under the same conditions, but instead maturation often leads to a loss of freshness). During wine aging, glycosides of terpenic alcohols and

some esters of carboxylic acids hydrolyse. Aldehydes are oxidised to carboxylic acids and these are partially esterified, the content of degradation products of carotenoids called norisoprenoids (such as vitispirane and others) increases and tannins polymerise.

Wines infected with either lactic acid bacteria (particularly heterofermentative strains) or *Dekkera*/*Brettanomyces* yeast can potentially produce mousy off-flavor for which 2-ethyltetrahydropyridine, 2-acetyltetrahydopyridine and 2-acetylpyrroline are responsible. The impact odorants contributing to mushroom off-flavour in wines are oct-1-en-3-one and non-1-en-3-one.

8.2.12.8.3 Spirits

In addition to ethanol, distillates contain a large number of volatile compounds, which either come from raw materials or are produced during fermentation. Other flavour-active components are formed during storage and maturation by reactions of the individual components of the distillates. Their content varies according to the quality of raw material, technology and depends on a number of other factors. Many aromatic compounds are fundamental to the final product; additional components have little importance or may have no importance at all. The main components are ethanol, methanol and higher alcohols. The total content of methanol and higher alcohols in whisky is usually about 1 g/l, and in brandy 1.5 g/l. Other compounds include carbonyl compounds and acetals, of which acetaldehyde and 1,1-diethoxyethane are the major components. The total content of carboxylic acids in whisky ranges from 100 (in Scotch whisky) to 400 (in Bourbon whisky) mg/l, of which the main component (amounting 40–95% of the total acidity) is acetic acid. The characteristic component of rum is 2-ethyl-3-methylbutanoic acid. Fatty acid esters of lower aliphatic alcohols are particularly important, as they carry a fruity flavour (especially in apricot fruit spirits and rum). The main ester is always ethyl acetate. Ethyl esters of higher fatty acids (up to ethyl palmitate) carry the soapy flavour of some spirits, such as whisky and brandy. Lactones, phenols (e.g. in whisky), terpenoids, nitrogen heterocycles and many other compounds also play important roles.

For example, the main odour-active components of Bourbon whisky are aldehydes, the most important of which are 3-methylbutanal, 2-methylbutanal, 2-methylpropanal and (2*E*,4*E*)-deca-2,4-dienal. Important components are also ethyl esters, such as butanoate, 2-methylpropanoate, (*S*)-2-methylbutanoate, 3-methylbutanoate, hexanoate and octanoate, and lactones, especially (3*S*,4*S*)-whisky lactone, δ-nonalactone and γ-decalactone, some phenols (vanillin and eugenol) and (*E*)-β-damascenone, which is a degradation product of carotenoids.

8.2.12.9 Additional commodities

8.2.12.9.1 Tea

The amount of black tea (*Camellia sinensis*, Theaceae) volatiles is about 0.01 to 0.02% of dry matter. Black tea contains about 4–5 times the number of aromatic compounds of green tea. Of the more than 300 known substances in tea, only certain compounds are important for the aroma. The basic aroma consists mainly of regular compounds having green odour, such as (*Z*)-hex-3-en-1-ol, (*E*)-hex-2-enal and hexanal, usually present are 3-methylbutan-1-ol, 2-phenylethanol, methyl salicylate, phenol and guaiacol. In Darjeeling tea, an important component is also (2*E*,4*E*,6*Z*)-nona-2,4,6-trienal, 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) and vanillin. Critical terpenoids and sensory active degradation products of carotenoids include α-terpineol, linalool, nerol, linalooloxides, β-damascone, (*E*)-β-damascenone, β-ionone, dihydroactindiolide and theaspiran and its derivatives, such as theaspiron and hydroxytheaspiran. The last compound is a very important component of the aroma of tea (its odour threshold concentration is about 0.2 μg/l in water). It constitutes about 1% of volatile substances. Compared with black tea, green tea contains lower amounts of linalool, linalool oxides and some other compounds, but higher amounts of nerolidol and β-ionone. A significant compound is 3-methylnonan-2,4-dione.

8.2.12.9.2 Coffee

Volatile components constitute about 0.1% of roasted coffee by weight (*Coffea* species, Rubiaceae), and more than 200 substances have been shown in green coffee. More than 800 compounds are known to make up the aroma of roasted coffee. Of these, only about 60 compounds have a significant role in the coffee aroma. Especially typical are a large number of heterocyclic compounds, mainly furans, pyrroles, indoles, pyridines, quinolines, pyrazines, quinoxalines, thiophenes, thiazoles and oxazoles, which arise in caramelisation and the Maillard reaction during coffee roasting. In addition to heterocyclic products, other important volatiles are also some aliphatic compounds (hydrocarbons, alcohols, carbonyl compounds, carboxylic acids, esters, aliphatic sulfur and nitrogen compounds), alicyclic compounds (especially ketones) and aromatic compounds (hydrocarbons, alcohols, phenols, carbonyl compounds and esters).

The aroma of coffee is very complex and virtually none of the above mentioned constituents provides the typical aroma by itself. Exceptions are 2-furanmethanethiol and 5-methyl-2-furanmethanethiol that have odours reminiscent of coffee. In freshly roasted coffee, 2-furanmethanethiol is present in an amount ranging from 0.01 to 0.5 μg/kg. A highly efficient sulfur-containing substance is also 2-methyl-3-furanthiol arising from thiamine. An important heterocyclic compound is 2-acetyl-4-methylthiazole and diterpene kahweofuran.

Significant aliphatic sulfur compounds are methional, 3-methylbut-2-ene-1-thiol, 3-mercapto-3-methylbutan-1-ol (**8-124**), its ester 3-mercapto-3-methylbutyl formate, methanethiol and dimethyltrisulfide. 3-Mercapto-3-methyl-1-ol also occurs in passion fruit and blackcurrant, and as a putative cat pheromone in cat urine, where it is formed as a degradation product of amino acid L-felinine (see Section 2.2.1.2.2). Of more than 70 known pyrazines, the most important compounds in roasted coffee are isopropylpyrazine, 2-isobutyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2,6-dimethyl-3-vinylpyrazine and 2-ethyl-6-methyl-3-vinylpyrazine. Pyridine and its alkyl derivatives and bicyclic pyridines have a negative impact on the quality of coffee aroma. Important aromatic

compounds include some furans and pyrans, especially γ-lactones, such as 3-hydroxy-4,5-dimethyl-(5*H*)-furan-2-one (sotolon), 5-ethyl-3-hydroxy-4-methyl-(5*H*)-furan-2-one (abhexon), as well as furan-2-carbaldehyde and maltol. Important phenols are vanillin, 4-vinylguaiacol and 4-ethylguaiacol, important carbonyl compounds include (*E*)-β-damascenone, biacetyl and pentane-2,3-dione, some Strecker aldehydes, such as acetaldehyde, 2-methylbutanal, 3-methylbutanal, propanal, (*E*)-non-2-enal and 2-hydroxy-3-methylcyclopent-2-en-1-one, known as cyclotene.

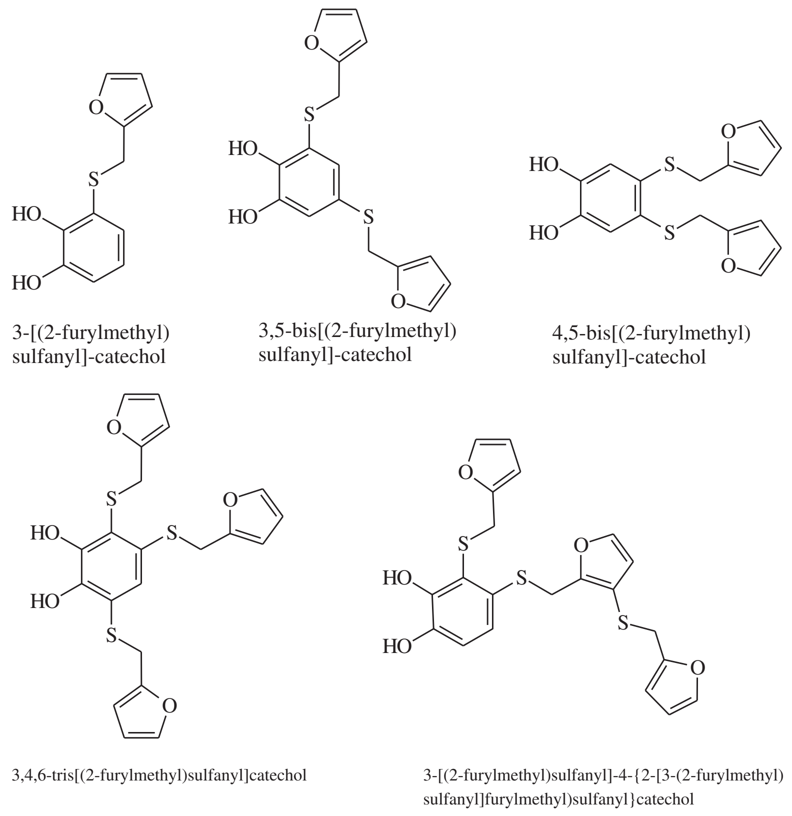
During storage of raw coffee beans atypical odours may develop, which are suggested to influence the aroma of coffee beverages. The compounds responsible for the atypical odour include (*E*)-β-damascenone with cooked apple-like odour, 2-methoxy-4-vinylphenol with clove-like odour and methyl 2-methyl and methyl 3-methylbutanoates showing fruity odour. The reduction of the water content in combination with lower temperatures are suggested to avoid aroma changes in raw coffee beans during storage.

Components of roasted coffee aroma, especially sulfur compounds, are easily oxidised, and ground roasted coffee kept in storage soon loses its typical aroma due to exposure to air. In order to retain the aroma, it is necessary to store it in impermeable containers under an inert atmosphere. Reactions of the odour-active thiols, such as 2-furanmethanethiol, with phenols generated from chlorogenic acid degradation products are responsible for the rapid aroma staling of coffee beverages. Examples of such reaction products are conjugates of catechol (pyrocatechol) with one, two and three molecule of 2-furanmethanethiol (**8-200**). Thiols also react with cysteine bound in proteins through thioether bond formation.

8.2.12.9.3 Cocoa and chocolate

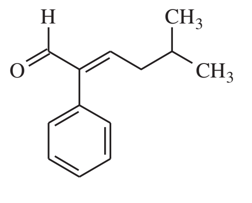
Fresh cocoa beans, the seeds of cacao tree (*Theobroma cacao*, Sterculiaceae), have a sour odour and a sour, bitter and astringent taste. The main contributors to the bitter taste are purine alkaloids. The astringent taste of cocoa products is mainly influenced by oligomeric procyanidins, flavan-3-ol-*C*-glycopyranosides (see Section 8.3.6.2.2) and *N*-phenylpropenoyl amino acids (see Section 2.2.1.2.1).

The formation of the characteristic aroma of cocoa depends on a number of factors, such as good harvest, fermentation, drying and roasting of cocoa beans. Precursors of aromatic compounds are produced mainly by anaerobic fermentation of cocoa beans. The aroma of roasted beans is created largely by the Maillard reaction and caramelisation, and is represented by more than 400 compounds. The major components are aldehydes, sulfides, heterocyclic compounds, carboxylic acids and terpenoids. Important aldehydes are 2-methylpropanal and 2-methylbutanal, which recalls the smell of cocoa and malt, and (*E*)-2-phenyl-5-methylhex-2-enal (**8-201**), which resembles chocolate. The latter aldehyde is formed by aldol condensation of another important aroma component, 3-methylbutanal with phenylacetaldehyde, and dehydration of the aldolisation product. Linalool and 2-phenylethanol

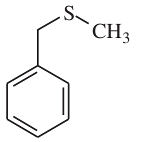


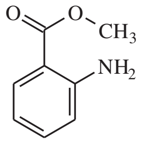
8-200, conjugates of catechol and 2-furylmethanethiol

carry sweet and floral scents, and 2-acetylpyridine has a roasted smell. Also important as aroma constituents are sulfides, especially dimethylsulfide, dimethyltrisulfide and benzyl methyl sulfide (**8-202**); among the other compounds, 2- and 3-methylbutyric acids, methyl anthranilate have a greater significance (**8-203**), and some Maillard reaction products, such as 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) and maltol, the smell of which resembles caramel, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine resembling aroma of fried potato chips, 2,4,5-trimethyloxazole and 4-methyl-5-vinylthiazole. A mixture of 24 compounds are reportedly necessary to simulate cocoa aroma, as with coffee aroma.



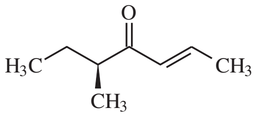
8-201, (*E*)-2-phenyl-5-methylhex-2-enal





8-202, benzyl methyl sulfide **8-203** methyl anthranilate

The main (key) components of chocolate aroma are C_5 carboxylic acids with a sweet smell (2-methylbutyric), lactones (γ-lactone with a sweet, peachy aroma), aldehydes (such as isovaleraldehyde with a sharp odour resembling malt), (*E*)-non-2-enal (with green and tallowy smell), (2*E*,4*E*)-nona-2,4-dienal and (2*E*,4*E*)-deca-2,4-dienal (with oily smell resembling fried foods), ketones, such as oct-1-en-3-one (with the mushroom-like smell), (2*E*,5*S*)- and (2*E*,5*R*)-5-methyl-hept-2-en-4-one (known as filbertone, which has the odour of hazelnuts, **8-204**), 2-ethyl-3,5-dimethylpyrazine (with a smell reminiscent of fried potato chips), 2-ethyl-3,6-dimethylpyrazine (with the smell of nuts with earthy tones) and methyl 2-methyl-3-furyl disulfide with meaty and sulfur smell.



8-204, (2*E*,5*S*)-5-methyl-hept-2-en-4-one

8.2.12.9.4 Peanuts

Unroasted peanut seeds (*Arachis hypogaea*, Fabaceae) contain about 75 volatiles, and in roasted seeds around 320 volatiles have been identified. The compounds of the greatest importance for the aroma of raw seeds are 2-isopropyl-3-methoxypyrazine (earthy and pea-like), 2-isobutyl-3-methoxypyrazine (bell pepper-like and earthy), and (*E*)-4,5-epoxy-dec-2-enal (metallic). Aroma components of roasted seeds include 2-acetyl-1-pyrroline, 2-propionyl-1-pyrroline, 6-acetyl-1,2,3,4-tetrahydropyridine, its tautomer 6-acetyl-2,3,4,5-tetrahydropyridine and 2-acetylpyridine (roasted aroma), 4-hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol) with a somewhat caramel odour, oct-1-en-3-one with a mushroom-like odour, 2-isopropyl-3-methoxypyrazine (pea-like odour), 2-isobutyl-3-methoxypyrazine (resembling a bell pepper flavour), methional (resembling cooked potato) and phenylacetaldehyde and phenylacetic acid with a sweet odour resembling honey). Important aldehydes formed by oxidation of fatty acids include (2*E*,4*E*)-deca-2,4-dienal (fried foods odour), (*E*)-4,5-epoxydec-2-enal (metallic odour), (*E*)-non-2-enal and (2*E*,4*E*)-nona-2,4-dienal (oily odour).

8.2.12.9.5 Honey

From more than 100 aroma components identified in different types of honey, the most important compounds are esters of aliphatic and aromatic acids, aldehydes, ketones and alcohols. Significant compounds are esters of phenylacetic acid and phenylacetaldehyde with strong honey-like odour. The origin of the honey also has a big influence.

8.2.12.9.6 Vinegar

The flavour of wine vinegar depends on the constituents formed during the fermentation of the wine and during the storage or the aging. Vinegar made from ethanol contains almost exclusively acetic acid, ethanol and ethyl acetate, which are the result of non-enzymatic esterification of acetic acid with ethanol during storage. In addition to the characteristic substances of the plant material, wine vinegars and vinegars made from fruit wines contain fermentation products, such as acetic, 3-methylbutyric, 2-methylbutyric and butyric acids, 2-phenylethanol, acetoin, biacetyl and fusel oil alcohols (mixture of 2- and 3-methylbutan-1-ol).

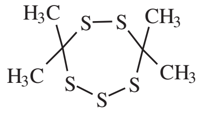
8.2.12.9.7 Mushrooms

Most types of wild and cultivated edible mushrooms of the phyla Basidiomycota (club fungi) and Ascomycota (sac fungi), which cover most of the so-called higher fungi, contain as the key compound alcohol (*R*)-oct-1-en-3-ol, which is produced by enzymatic oxidation of linoleic acid. Oct-1-en-3-ol is accompanied by a number of other compounds. For example, the components of common mushroom (*Agaricus bisporus*) aroma are the following alcohols and carbonyl compounds: 3-methylbutan-1-ol, (*R*)-octan-3-ol, (3*R*,5*Z*)-octa-1,5-dien-3-ol, (*Z*)-oct-2-en-l-ol (having a weak mushroom odour), octan-3-one, benzyl alcohol, benzaldehyde and furan-2-carbaldehyde. Certain non-volatile compounds are important for the mushroom taste, especially free nucleotides.

Some other sensory active substances are formed during cooking. A particularly significant compound is oct-1-en-3-one, which has a metallic and faintly mushroom odour that is important in the transformation of raw mushroom smell to the smell of cooked mushrooms. The aromatic components of dried mushrooms are numerous carboxylic acids, their lactones, aliphatic sulfur

compounds and heterocyclic compounds (especially pyrazines and pyrroles) formed as products of the Maillard reaction.

For the typical aroma of some species of fungi, other substances are significant. Aliphatic sulfur compounds methyl (methylthio)methyl disulfide (**8-186**), bis(methylthio)methane, tris(methylthio)methane and dimethyl sulfide contribute significantly to the typical aroma of truffles (*Tuber brumale*, *T. melanosporum* and *T. magnatum*). The musky odour of these mushrooms is derived from (3α,5α)-androst-16-en-3-ol, which is present in mushrooms at concentration of about 50 mg/kg. In China and Japan (and also in Europe) the widely used shiitake mushroom (*Lentinula edodes*), with a distinctive sulfurous aroma, has as a characteristic component 4,4,7,7-tetramethyl-1,2,3,5,6-pentathiepane, known as lenthionine (**8-205**). Lenthionine arises from lentinic acid by enzymatic reaction along with other sulfur compounds. The odour threshold concentration ranges from 0.3 to 0.5 mg/kg in water. The major constituents of the unpleasant smell of common stinkhorn (*Phallus impudicus*) are dimethyl disulfide and dimethyl trisulfide. Minor components are linalool, *trans*-β-ocimene and phenylacetaldehyde.



8-205, lenthionine

8.2.12.9.8 Spices

Fresh, dried or otherwise prepared parts of some plants have an intense, pleasant and characteristic odour and taste, and are therefore used as spices for food flavouring. The main constituents of the aroma of spices are various volatile mono- and sesquiterpenoids, in particular hydrocarbons, alcohols, aldehydes, ketones, esters and ethers (Table 8.32). The total content of essential oils is in the tenths to units of percent. Some spices also contain components with characteristic spicy, sharp and burning taste and important pigments.

8.2.13 Physiology and nutrition

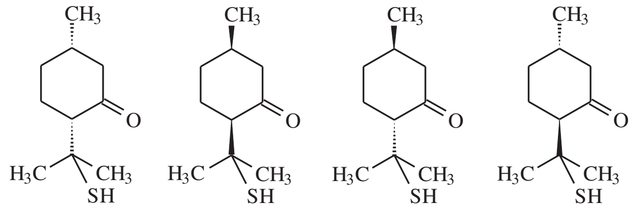
8.2.13.1 Organoleptic properties

8.2.13.1.1 Structure of odour-active compounds

Characteristic organoleptic properties of odoriferous substances are related to their structure and their stereochemistry. Small changes in the structure of molecules often lead to drastic changes in the quality and quantity of sensory perception. Structural analogues of compounds or structural isomers (positional isomers as well as functional isomers) therefore often exhibit different organoleptic properties. Odour-active substances are mostly chiral molecules, and the individual enantiomers or diastereoisomers have different organoleptic properties. Smell is a phenomenon associated with the elementary composition of substances, their spatial arrangement and, in unsaturated compounds, with molecular geometry. Reactions in which these compounds arise are enantioselective and diastereoselective.

For example, the pleasant smell of α-terpineol (**8-19**) resembles lilac flowers, but the substitution of oxygen for sulfur turns this into a very aggressive smell of *p*-menthene-8-thiol (**8-188**), which only at very low concentrations (below 1 μg/kg) recalls the scent of grapefruits for which (*R*)-*p*-mentha-1-en-8-thiol is a key component. The individual enantiomers of α-terpineol and 1-*p*-menthene-8-thiol also have different odours. (+)-(*R*)-α-terpineol (**8-19**) has a typical heavy smell of lilac flowers, (−)-(*S*)-α-terpineol (**8-19**) has smell of coniferous trees and tar, the natural isomer (+)-(*R*)-1-*p*-menthene-8-thiol (**8-188**) has the aroma of grapefruit, but the (*S*)-enantiomer (**8-188**) has a weak and non-specific flavour.

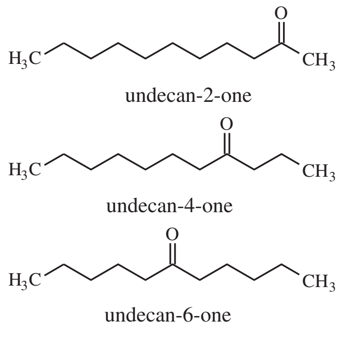
Blackcurrant aroma is reminiscent of only one diastereoisomer of *p*-menthan-8-ol-3-one, that is (1*S*,4*R*)-*p*-menthan-8-ol-3-one (**8-206**). This compound is also a characteristic component of blackcurrant leaves and fruits. In contrast, (1*R*,4*S*)-*p*-menthan-8-ol-3-one (**8-206**) has an unpleasant smell resembling burnt rubber and mercaptans, (1*R*,4*R*)-*p*-menthan-8-ol-3-one (**8-206**) smells like onion and has a weak fruity odour, and (1*S*,4*S*)-*p*-menthan-8-ol-3-one (**8-206**) has a similar but stronger smell of tropical fruits and a faint sulfur smell.



(1*S*,4*R*)-isomer (1*R*,4*S*)-isomer (1*R*,4*R*)-isomer (1*S*,4*S*)-isomer

8-206, *p*-menthan-8-ol-3-one isomers

The long-chain ketone undecan-6-one (**8-207**) has a strong fruity smell, but its positional isomer undecan-2-one (**8-207**) smell is reminiscent of rue (*Ruta graveolens*, Rutaceae). The smell of undecan-4-one (**8-207**) lies between these two extremes.



8-207, undecanone isomers

Table 8.32 Basic composition of common spices.

Latin name	Main components of essential oil[a]
Apiaceae	
Anethum graveolens Dill	**(+)-Carvone, limonene, (+)-dihydrocarvone**, (−)-carveol (seeds); **α-phellandrene, (+)-dill ether** (haulm)
Carum carvi Caraway	**(+)-Carvone, (+)-limonene**, α-pinene, α-phellandrene, dihydrocarvyl acetate, 1,8-cineole, linalool
Coriandrum sativum Coriander	**(+)-Linalool**, linalyl acetate, citral, α-pinene, β-pinen, α-phellandrene, α-terpinene, *p*-cymene, decan-1-ol, geraniol, borneol
Cuminum cyminum Cumin	**Cuminaldehyde**, **α-pinene**, **β-pinene**, *p*-cymene, 2-ethoxy-3-isopropyl-, 2-methoxy-3-*sec*-butylpyrazine, 2-methoxy-3-methylpyrazine
Foeniculum vulgare Fennel	(*E*)-**Anethole**, **fenchol**, α-phellandrene, (+)-limonene, estragol, camphene
Levisticum officinale Lovage	3-Butylidene-4,5-dihydrophthalide (ligustilide), other phthalides, α-terpinyl acetate, α-terpineol, carveol, carvacrol, coumarin
Pimpinella anisum Anise	**(*E*)-Anethole**, estragol, 4-methoxyacetofenone, acetaldehyde, 4-isopropylbenzaldehyde, 1,8-cineole
Asteraceae	
Artemisia dracunculus Tarragon	**Estragol**, 4-methoxycinnamaldehyde, 5,7-dimethoxycoumarin, anethole, α-pinene, myrcene
Cupressaceae	
Juniperus communis Common juniper	**α-Pinene**, **myrcene**, **β-pinene**, (+)-limonene, *p*-cymene, camphene, terpin-1-en-4-ol, α-terpineol, borneol, β-cadinene, sabinene
Illiciaceae	
Illicium verum Star anise	**(*E*)-Anethole**, α-phellandrene, α-pinene, β-cadinene, Δ^3-carene, *p*-cymene, dipentene, α-terpineol, estragol
Lamiaceae	
Mentha piperita Peppermint	**(−)-Menthol, (−)-menthone, (−)-menthyl acetate**, (+)-menthofuran, α-pinene, α-phellandrene, β-caryophyllene
Mentha spicata Spearmint	**(−)-Carvone, limonene**, dihydrocarvone, sabinene hydrate, (−)-menthone
Ocimum basilicum Basil	**Estragol**, **linalool**, 1,8-cineole, eugenol, methyl cinnamate, nerol, linalyl acetate
Origanum majorana Marjoran	**Terpin-1-en-4-ol**, **(*Z*)-sabinene hydrate**, (*E*)-sabinene hydrate, **1,8-cineole**, **estragol**, **α-terpineol**, **eugenol**
Origanum vulgare Oregano	Carvacrol, thymol, *p*-cymene, carvacryl methyl ether, linalool, α-pinene, bornyl acetate, camphor
Rosmarinus officinalis Rosemary	**1,8-Cineole**, **α-pinene**, **camphor**, camphene, borneol, bornyl acetate
Salvia officinalis Common sage	α-Thujone, β-thujone, camphor, 1,8-cineole, α-pinene, β-pinene, bornyl acetate, myrcene, borneol, linalyl acetate, β-ocimene
Thymus vulgaris Common thyme	**Thymol**, **carvacrol**, *p*-cymene, linalool, limonene, α-pinene, camphene, terpinene, β-caryophyllene, geraniol, carvacrol,
Lauraceae	
Cinnamomum verum True cinnamon	**Cinnamaldehyde**, **eugenol**, **safrol**, **linalool**, camphor

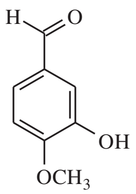
(*continued overleaf*)

Table 8.32 (*continued*)

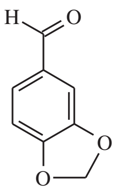
Latin name	Main components of essential oil[a]
Laurus nobilis Bay laurel	**1,8-Cineole, linalool**, eugenol, α-pinene, α-phellandrene, geraniol, γ-terpinene, *p*-cymene, β-caryophyllene, eugenyl acetate, camphene
Myristicaceae	
Myristica fragrans Nutmeg	**α-Pinene, β-pinene, sabinene, limonene**, 1,8-cineole, safrol, myristicin, γ-terpinene, terpinen-4-ol, eugenol, isoeugenol
Myrtaceae	
Syzygium aromaticum Clove	**Eugenol, β-caryophyllene**, eugenyl acetate, α-pinene, methyl benzoate, methyl salicylate, heptanol, nonanol, furfuryl alcohol
Pimenta dioica Allspice	**Eugenol**, 1,8-cineole, β-caryophyllene, methyleugenol, α-phellandrene
Piperaceae	
Piper nigrum Black pepper	**α-Pinene, β-pinene, sabinene, β-caryophyllene, Δ^3-carene, limonene**
Zingiberaceae	
Curcuma longa Turmeric	**(+)-*ar*-Turmerone, zingiberene**, 1,8-cineole, borneol, α-phellandrene
Elettaria cardamomum Cardamom	**1,8-Cineole, α-terpinyl acetate, (+)-limonene**, sabinene, borneol
Zingiber officinale Ginger	**(−)-Zingiberene, β-sesquiphellandrene, β-bisabolene**, citral, citronellyl acetate

[a]Components occurring in quantities higher than 10% are highlighted in bold.

Vanillin (**8-43**) has a typical smell of vanilla, isovanillin (**8-208**) does not resemble vanilla at all, and heliotropin (**8-209**) has a floral and spicy smell reminiscent of both vanillin and isovanillin.

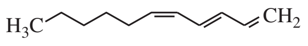


8-208, isovanillin

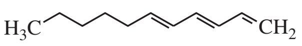


8-209, heliotropin

Similarly, different organoleptic properties are also found in *cis/trans*, respectively (*E*,*Z*)-isomeric substances. For example, hydrocarbon galbanolene, which is (3*E*,5*Z*)-undeca-1,3,5-triene (**8-210**) occurring in galbanum (aromatic resin of *Ferula gumosa*, Apiaceae), celery and parsley leaves, celery root and mandarin oil, has a green smell, but its *all-trans* isomer (3*E*,5*E*)-undeca-1,3,5-triene has an oily smell (**8-210**). (*Z*)-Isomers are generally considered natural and are therefore more acceptable, (*E*)-isomers are often associated with synthetic compounds and various off-flavours.



(3*E*,5*Z*)-undeca-1,3,5-triene



(3*E*,5*E*)-undeca-1,3,5-triene

8-210, undeca-1,3,5-triene isomers

Enantioselectivity and diastereoselectivity of substances is of clear importance for the biological activity of compounds, and plays a role in chemical communication systems in bacteria, algae, insects, fish and higher animals. It is not surprising, therefore, that the enantiomeric forms of compounds often show differences in smell (and taste).

Analysis of individual forms is also important to determine the authenticity of foods or essential oils. The presence of certain forms, such as the geometrical (*E*)-isomers, is often evaluated as an off-flavour. For example, (+)-(*R*)-limonene (**8-5**) is a natural component of citrus essential oils and has a citrus smell like oranges. The isomer (−)-(*S*)-limonene (**8-5**), which is the main component of essential oils of silver fir (*Abies alba*, Pinaceae), other conifers and various types of mints (*Mentha* spp.), has a smell reminiscent of turpentine. The alcohol (−)-(*R*)-linalool, also known as licareol (**8-18**) has a strong woody smell like lavender, but (+)-(*S*)-isomer, also known as coriandrol (**8-18**) has a sweet smell with lavender tones. Another alcohol (−)-(*R*)-oct-1-en-3-ol (**8-16**), has an intense smell of fresh mushrooms, but (+)-(*S*)-isomer (**8-16**) has a smell resembling vegetables with faint mushroom notes. The ketone (−)-(*R*)-carvone (**8-50**) has a mint-like smell (and is the main component of spearmint essential oil), while its isomer (+)-(*S*)-

carvone (**8-50**) smells like caraway and is a major component of caraway essential oil.

Menthol has three asymmetric carbon atoms in the molecule, so there exist four pairs of optical isomers, but only (−)-(1*R*,3*R*,4*S*)-menthol (**8-19**) has any practical significance. It has a sweet, fresh, minty smell and a cool, refreshing taste. In the other isomers the mint smell and cool taste are weaker, and the dominating characteristic is vegetable-like. The other three menthol isomers, (+)-neomenthol, (+)-isomenthol and (+)-neoisomenthol have certain significance, but further isomers have little importance.

The only isomer of nootkatone that resembles the odour of grapefruits is (+)-nootkatone (**8-52**), while the remaining isomers have a more woody and spicy smell.

8.2.13.1.2 Sensitivity to perception of odours

Sensitivity to odour perception is different for each person, and also depends on the physiological state of the organism, psychological conditions, pathological changes and other factors. Generally, women are more sensitive to odours than men. Both sexes show a maximum sensitivity at puberty, and sensitivity to the perception of odours is reduced after the 70th year of life, and drastically after the 80th year of life. The sensitivity is also lower during pregnancy, colds and some diseases. Smokers perceive some odours as less intense (e.g. odour of cloves and smell of thiols), but other odours more intensely (such as banana aroma).

Part of the population suffers from so-called specific **anosmia** (specific olfactory blindness), which is manifested by an inability to perceive certain odours. About 100 substances are known that are not perceived by a limited number of human beings. For example, 70.5% of women and 62.8% of men reportedly perceive (5α)-androst-16-en-3-one, while the scents of cloves and bananas are perceived by nearly 100% of the population. About 1.2% of the total population suffer from total anosmia, which is a condition in which people have no sense of smell. People with anosmia often also experience **ageusia**, the inability to taste, because smell also plays an important role in taste perception. Hyperosmia is an extremely sensitive sense of smell, while parosmia causes people to misinterpret smells, detecting something unpleasant when the odour is neutral or pleasant. In phantosmia (a sort of olfactory hallucination), people detect smells where there are none. Anosmia differs from temporary olfactory adaptation (fatigue), which is adaptation to the smell of an odoriferous substance after a very short time when the smell ceases.

The ability to perceive the odour of a particular substance also depends on its **odour threshold** (aroma, flavour) **value**, which is the concentration of a substance detectable by the sense of smell. The **odour detection threshold value** is the lowest concentration of a stimulus (odoriferous substance), which can be detected in comparison with an environment that does not contain this substance. The **odour recognition threshold value** is the concentration at which a substance can not only be detected, but also recognised. It corresponds to a concentration that allows identification of the odour quality of a substance, which is usually higher than the odour detection threshold. Both values are measures of the odour intensity, but depend considerably on the environment, solubility, partition coefficients between air and water (oil) and some other factors. For example, values measured in air are typically several orders of magnitude lower than the values measured above aqueous solutions. The substance with a high odour threshold value must be present in foods in higher concentrations than substance with a low odour threshold value, otherwise its smell is imperceptible. The measure of whether the substance acts as an odour-active substance is its **odour unit**, its actual concentration divided by odour threshold concentration.

The presence of lipids, proteins, carbohydrates and other substances significantly influences the retention of aromatic compounds in foods and has an effect on their odour intensity and quality (e.g. the odour threshold values of non-polar substances measured in water are usually lower than the values measured in

Table 8.33 Odour detection threshold values of most powerful odoriferous substances (in μg/l in water at 20 °C).

Compound	Occurrence	Odour detection threshold
5-Ethyl-3-hydroxy-4-methyl-5*H*-furan-2-one (abhexon)	Acid protein hydrolysates	0.000 000 01
(*S*)-Butylphthalide	Celery, celeriac	0.000 000 01
3-Hydroxy-4,5-dimethyl-5*H*-furan-2-one (sotolon)	Fenugreek, caramel, brown sugar, maple syrup	0.000 001
Bis(2-methyl-3-furyl)disulfide	Thiamine photolytic product	0.000 002
2-Isobutyl-3-methoxypyrazine	Bell pepper	0.000 002
2,4,6-Trichloroanisole	Cork bottle stoppers (off-flavour)	0.000 003
(+)-(*R*)-*p*-Menth-1-en-8-thiol	Grapefruit juice	0.000 01–0.000 02
Mesifuran	Fruits	0.000 03
Furfuryl mercaptan	Roasted coffee	0.005–0.01
Skatole	Pork meat (off-flavour)	0.006
β-Damascenone	Apples, alcoholic beverages	0.009

emulsions or fats). The effects known as **synergism** and **antagonism** play important roles in mixtures of odoriferous substances, causing higher (in synergism) or lower (in antagonism) response to the mixture of compounds than that expected from simple additivity among the components. For example, the odour of butyric acid is more intense in the presence of isovaleric acid and less intense in the presence of acetic acid. Antagonistic odorants are, for example, eugenol and methyl isoeugenol.

Table 8.33 displays the odour threshold values of some intensely odoriferous substances. The odour threshold values of selected common odoriferous substances are listed in Table 8.34 and Table 8.35.

8.2.13.2 Physiological effects

Odoriferous substances and their mixtures (e.g. essential oils) show a number of beneficial effects for which they have found use as pharmaceuticals, pharmaceutical ingredients and food additives. Bactericidal and anti-inflammatory effects are seen in borneol, eugenol, pinenes, camphor, thymol and menthol, cholinolytic (spasmolytic) effects preventing a drop in blood pressure and suppressing the secretory activity of various organs are found in camphor, pinenes and camphene, and analeptic effects (stimulating activity of circulation and respiratory system) are seen in camphene. The essential oils of many spices (such as marjoram, sage, thyme and many others) exhibit antioxidant effects, and therefore find use as natural antioxidants of fats.

Some terpenoids and other aromatic substances also show a variety of toxic effects, and their content in foods is therefore restricted by legislation in many countries.[5]

The monoterpenes α-thujone, known as (−)-thujone, and β-thujone, known as (+)-isothujone (**8-50**), are the dominant components of various essential oils of plants of the genus *Artemisia* (Asteraceae) that are referred to under various names, such as mugwort, sagebrush, sagewort, wormwood, tarragon and others. The content of essential oils ranges from 0.25 to 1.32%, in which thujones are present in amounts of 3–12%. Thujones also occur in the essential oil of sage (*Salvia officinalis*, Lamiaceae) and tansy (*Tanacetum vulgare*, Asteraceae), while in lower levels thujones are found in yarrow (*Achillea millefolium*, Asteraceae) and other plants. All these plants are used as medicinal herbs or find use as bittering agents in alcoholic beverages. For example, tansy is used for bittering Chartreuse liquor and wormwood for absinthe. α-Thujone is perhaps best known as the active ingredient of the green liquor absinthe, which was a very popular French drink in the 1800s and gained considerable notoriety as a preferred liqueur of artists and writers. For example, by 1910, the French were drinking 36 million litres of absinthe per year. Although absinthe became an epidemic health problem, leading to a ban in France in 1915, its use continues on a small scale, either legally or illicitly. The acute toxicity of α-thujone can be attributed to blocking the γ-aminobutyric acid (GABA)-gated chloride channel. Both thujones show chronic neurotoxicity manifested by hallucinations, hyperexcitability, sleeplessness, tremors, convulsions and later damage of the cerebral cortex. Manifestations of chronic toxicity are called absinthism, the disorder associated with the habitual abuse of absinthe. The thujones content is therefore restricted. For example, the preban absinthes produced in the period 1895–1914 contained 0.8–7.7 mg/l α-thujone and 0.6–39.5 mg/l β-thujone, while the concentrations of these two substances in modern legal absinthes (produced in Europe in 2003–2006) were 1.4–5.6 and 0.7–67.6 mg/l, respectively.

Toxic effects have are also been found for (+)-(*R*)-*p*-mentha-4(8)-en-3-one, known as (+)-pulegone (**8-50**), which is found in essential oils of different mint species, especially in squaw mint, also know as pennyroyal (*Mentha pulegium*, Lamiaceae), but also in other mint species. Mints are mostly used as carminative agents (anti-flatulence agents), spasmolytic agents (release spasms), stimulants of bile and gastric juices production and food flavouring. Pulegone occurring, for example, in peppermint oil exhibits neurotoxicity and relatively high hepatotoxicity leading to necrosis of liver cells. Its content in food is therefore restricted. The bitter sesquiterpene santonin (see Section 8.3.5.1.3) occurs mainly in the flowering tops of plants of the genus *Artemisia* and in their essential oils. Previously, these drugs were used as intestinal anthelmintic drugs. Santonin promotes chromatopsia (an abnormal condition, in which all objects appear in purple colour). Its level in food is therefore legally restricted. Carcinogenic effects are shown by some substituted alkenylbenzenes (e.g. prop-1-en-1-ylbenzenes and prop-2-en-1-ylbenzenes), such as safrole (**8-34**) and methyleugenol (**8-33**) that occur in several essential oils. Myristicin, found in seed essential oils of some root vegetables (such as carrots), is a psychomimetic substance having narcotic and hallucinogenic effects similar to the effects of ethanol. It also displays antimicrobial effects. β-Asarone (**8-33**), occurring mainly in wild ginger (*Asarum europaeum*), has chemosterilation effects. Another aromatic substance, the use of which is restricted, is coumarin (**8-99**), which occurs, for example in tonka beans (*Dipteryx odorata*, Fabaceae) and many other plants. Coumarin has hepatotoxic effects, but certain anti-carcinogenic properties and some positive effects in the treatment of varicose vein syndrome (such as rutin and hypericin) have also been demonstrated.

8.2.14 Production and use

Flavourings are products that are intended to be added to food in order to impart or modify odour and/or taste. They are made or consist of the following categories: flavouring substances, flavouring preparations, thermal process flavourings, smoke flavourings, flavour precursors or other flavourings or mixtures thereof. Food flavourings are derived from natural materials or produced synthetically. It is estimated that of the food flavourings used in developed countries, about 75% of substances are natural compounds and the rest are synthetic compounds. About 99% of synthetic substances

[5] Influence of odoriferous substances on human health is concerned with the organisation IFRA (International Fragrance Association) and other organisations. In the Code GRAS (Generally Regarded as Safe) published by FEMA (Flavour and Extract Manufacturer's Association) and in the Code published by CoE (Council of Europe), non-toxic flavour-active substances are allocated numbers (FEMA numbers or CoE numbers), as well as Chemical Abstracts Service (CAS numbers).

Table 8.34 Odour detection threshold values of aliphatic and aromatic substances in mg/l in water at 20 °C.

Compounds	Occurrence	Odour detection threshold
Hydrocarbons		
α-Humulene	Essential oils (hops)	0.1
Limonene	Essential oils (citrus fruits)	0.01
Aldehydes		
Acetaldehyde	Widespread (alcoholic beverages)	0.009
Benzaldehyde	Essential oils (bitter almonds)	0.4
(2*E*,4*E*)-Deca-2,4-dienal	Rancid fats, some essential oils	0.0001
Hexanal	Essential oils, rancid fats	0.005[a]
(*E*)-Non-2-enal	Essential oils (carrots)	0.0001
Phenylacetaldehyde	Widespread (phenylalanine degradation)	0.04
α-Sinensal	Essential oils (oranges)	0.000 1
Vanillin	Essential oils (vanilla)	0.02
Alcohols		
Ethanol	Alcoholic beverages	100
Geraniol	Essential oils, widespread	0.1
Hexan-1-ol	Essential oils, alcoholic beverages, widespread	0.8–1
(*Z*)-Hex-3-en-1-ol	Fruits, vegetables	70
(*E*)-Hex-3-en-1-ol	Fruits, vegetables	2
Linalool	Essential oils, widespread	0.1
Oct-1-en-3-ol	Mushrooms, molds	0.001
Thymol	Essential oils (thyme)	0.1
Ketones		
Biacetyl	Fermentation product (butter)	2
Cyclotene	Roasted products containing sugar	0.3
Oct-1-en-3-one	Mushrooms, moulds	0.001
Acids		
Acetic acid	Vinigar	25
Butyric acid	Rancid butter	0.2
Phenylacetic acid	Essential oils, cocoa, fruits	10
Esters		
Ethyl acetate	Widespread (alcoholic beverages)	5–25
Ethyl butyrate	Fruits, alcoholic beverages	0.001
Ethyl myristate	Alcoholic beverages	2
Geranyl acetate	Widespread (essential oils)	0.01
Lactones		
γ-Decalactone	Fruits, dairy products	0.01
γ-Dodecalactone	Fruits, dairy products	0.01
δ-Decalactone	Fruits, dairy products	0.1
δ-Dodecalactone	Fruits, dairy products	0.2

(*continued overleaf*)

Table 8.34 (*continued*)

Compounds	Occurrence	Odour detection threshold
Aliphatic sulfur compounds		
Dimethyl disulfide	Widespread (vegetables and meat)	0.01
Dimethyl sulfide	Widespread (vegetables and meat)	0.000 1
Dimethyl trisulfide	Widespread (vegetables, meat, chocolate)	0.000 01
Ethanethiol	Widespread (vegetables and meat)	0.000 01
3-Mercaptobutan-2-one	Widespread (vegetables and meat)	0.003
3-Mercaptopentan-2-one	Widespread (vegetables and meat)	0.001
2-Mercaptopentan-3-one	Widespread (vegetables and meat)	0.001
Methanethiol	Widespread (vegetables and meat)	0.000 02
Methional	Widespread (potatoes, vegetables and meat)	0.000 2
Sulfane	Widespread (vegetables and meat)	0.01
3-Methylbut-2-en-1-thiol	Beer (off-flavour)	0.001
Amines		
Trimethylamine	Fish, eggs (off-flavour)	2

[a] The odour detection threshold value of non-polar substances in oil is different. For example, for hexanal 0.12 mg/kg, for (*E*)-non-2-enal 0.15 mg/kg, for (2*E*,4*E*)-deca-2,4-dienal 0.135 mg/kg and for phenylacetaldehyde 0.022 mg/kg.

are substances occurring in nature (nature identical substances) and 1% of substances are artificial, synthetic flavourings.

The most important materials for the production of natural food flavourings (but also perfume compositions for cosmetic purposes) are **essential oils**, **oleoresins**, extracts, juices, pulps and distillates. They are obtained almost exclusively from plant materials, fresh or dried plants or parts of plants otherwise prepared for drugs. Materials called **resinoids** are primarily used for the fixation of fragrance compositions in cosmetics and only occasionally in foods. Drugs of animal origin are not used for the production of food flavourings, in contrast to cosmetic products.

8.2.14.1 Essential oils

Essential oils are complex mixtures of volatile substances contained in natural plant materials. They are obtained from different parts of plants, their flowers (such as jasmine oil), flowering stalks or straw (such as mint and thyme oils), fruits or seeds (such as cumin, pepper and juniper oils), fruit pericarp (such as citrus oils), wood (sandal oil) leaves (bay oil), bulbs (such as garlic oil), rhizomes (such as sweet flag and turmeric oils) and roots (such as gentian oil). Essential oils are obtained by three basic procedures or their combinations:

- mostly by distillation with water vapour and separation of the oil layer in the distillate (mainly oils from the seeds, stems, leaves, wood and roots); on extraction of the distillation waters an additional portion of oil, usually of lower quality is obtained;
- by extraction with non-polar solvents, such as petrol and petroleum ether (mainly oils of flowers), the extract obtained is called **miscella**; Freons are used as solvents (the trade name refers to several chlorofluorocarbons) and carbon dioxide at supercritical pressures, extraction with animal fats (such as pork lard) is now used less frequently, the procedure is called **enfleurage** (mostly for volatile oils of flowers)
- by pressing and separation of the oil layer (mainly oils of citrus fruit peels).

Steam distillation, extraction with non-polar solvents, pressing and evaporation of solvent produce an essential oil known as a **concrete**, which is a mixture of essential oil, various lipophilic substances and mainly waxes. The accompanying substances are separated by dissolution in hot ethanol, freezing and filtration and the essential oil thus obtained is **absolute**.

Most essential oils contain a significant proportion of terpenes (monoterpenic and sesquiterpenic hydrocarbons). For example, their level in some citrus essential oils is 95% or more. These substances are not usually essential to the smell and aroma character of essential oils, as the most important odoriferous compounds are alcohols, aldehydes, ketones, esters and other compounds. Furthermore, terpenic hydrocarbons are a reason for the limited solubility of essential oils in diluted ethanol and are often the cause of deterioration of essential oils that easily oxidise or polymerise. By removing hydrocarbons from essential oils, concentrates are obtained. Monoterpene-free or sesquiterpene-free essential

Table 8.35 Odour detection threshold values of heterocyclic substances in mg/l in water at 20 °C.

Compound	Occurrence	Odour detection threshold
Oxygen heterocycles		
Furan-2-carbaldehyde	Widespread, degradation of pentoses	3
4-Hydroxy-2,5-dimethyl-2*H*-furan-3-one (furaneol)	Widespread (meat, milk, cheeses, bread, fruits, vegetables)	0.03
Maltol	Caramel, bread	35
Sulfur heterocycles		
5-Acetyl-2,3-dihydro-1,4-thiazine	Coffee, meat	0.001
2-Acetylthiazole	Widespread in cooked foods	0.01
3,4-Dimethylthiophene	Roasted onion	0.001
2-Isobutylthiazole	Fresh tomatoes	0.002–0.03
2-Furanmethanethiol	Coffee, meat	0.000 005
2-Methyl-3-furanthiol	Coffee, meat	0.000 01
2-Methyl-3-thiophenethiol	Coffee, meat	0.000 02
Nitrogen heterocycles		
Acetylpyrazine	Widespread in cooked foods	0.1
2-Acetylpyridine	Widespread in cooked foods	0.02
2-Acetyl-1-pyrroline	Bread, cereal products	0.0001
2-Acetyl-1,2,3,4-tetrahydropyridine	Bread, cereal products	0.001
2,5-Dimethylpyrazine	Widespread in cooked foods	0.8
2,6-Dimethylpyrazine	Widespread in cooked foods	0.2
2-Methyl-3-ethylpyrazine	Widespread in cooked foods	0.000 4

oils (that do not even contain monoterpenic hydrocarbons) are collectively called **deterpenic essential oils**. They are more concentrated than the starting oils, have a sensory character of the original oils and are more stable against autoxidation and suitable for products with a lower ethanol content. Deterpenation of essential oils is carried out by distillation, extraction, adsorption on suitable sorbents or by a combination of these procedures.

Essential oils obtained by cold pressing or extraction contain, in addition to volatile substances, low volatile and non-volatile substances, such as oleoresins. For example, the dry matter content in citrus essential oils is about 4%. The main components are flavonoids, such as naringin, or metabolites of bitter triterpenes of limonin type. Coumarins such as scopolaron are present at a level of 1.5%, along with furanocoumarins (psoralens), such as bergaptene and bergamottin.

8.2.14.2 Oleoresins

Oleoresins are the flavour extracts obtained by the solvent extraction of the ground spices or vegetables and solvent evaporation from the miscella. They are mixtures of essential oils, resins and other components that are extracted with organic polar (methanol, ethanol and isopropyl alcohol), non-polar solvents (hexane, petroleum ether and gasoline) or by supercritical carbon dioxide extraction. Oleoresins have the aroma of the original spices or vegetables as they contain essential oils in varying proportions, and possess attributes which contribute to the taste, such as pungency of black pepper and ginger oleoresins, and colour of turmeric and hot bell pepper oleoresins. For example, the essential oils of black pepper (Table 8.32) obtained by steam distillation contain as major components (about 20%) the terpenes α-pinene, sabinene and β-caryophyllene and in smaller amounts car-3-ene, limonene, β-pinene and other compounds. Oleoresin of black pepper obtained by extraction contains about 13–14% essentials oil and 50% of hot piperine and related non-volatile substances.

The oleoresins have some advantages over natural spices (Table 8.36), because of their low microbial contamination, uniformity in flavour, colour and pungency, easy storage and transport. Oleoresins as such or deposited on suitable media (such as starch or flour) have several applications such as in preparation of beverages, soup powders, confectionary, curries, noodles, sauces, canned meat and meat products.

Table 8.36 Characteristics of some common oleoresins.

Oleoresin	Essential oil (ml/kg)	Spice equivalent[a]	Oleoresin	Essential oil (ml/kg)	Spice equivalent[a]
Bay leaf	300	3.5	Allspice	600	4.5
Loves	700	6	Bell pepper	Traces[b, c]	10
Cumin	600	5	Black pepper	150[c, d]	4.5
Coriander	330	10	Cinnamon	160	5.5
Turmeric	Traces[b, e]	15	Sage	250	2
Marjoram	400	3	Vanilla	50[f]	5
Mace	500	7.5	Thyme	500	4
Nutmeg	800	6	Ginger	250[c, g]	4

[a]Spice equivalent is given by the number of weight units that have the same flavour as 100 units of natural spices.
[b]A minimum content of dyes is required.
[c]By sensory analysis is measured the sharpness (heat) of hot oleoresins, which is expressed in Scoville Heat Units.
[d]50% of piperine.
[e]At least 12.5% of curcumine.
[f]10% of vanillin.
[g]12% of hot ketones.

8.2.14.3 Resinoids

Resinoids are products obtained by the extraction of natural materials, mostly resins, gum resins and balsams, by solvents other than petroleum ether or petrol. Chlorinated hydrocarbons (dichloromethane and chloroform), acetone, methanol or ethanol are typically used for this. Resinoids have a characteristic smell, which due to the volatile substances (essential oils) present.

Resins are the products of various shrubs and trees, especially conifers. In addition to essential oils, resins contain resin acids, such as tricyclic diterpene abietic acid, resin alcohols (resinols), resin esters and other substances. Particularly well known is Chios mastic gum. Real mastic is only produced in the south part of Chios Island, and comes from the mastic tree (*Pistacia lentiscus* var. *chia*, Anacardiaceae). It is used to flavour alcoholic beverages (especially liqueurs and ouzo), wine, baked goods, chewing gum and some cosmetic products. The essential oil with a balsam-like odour has antiseptic properties as it contains α- and β-pinene as essential ingredients.

Gum resins are secreted by some plants. They contain essential oils, resins and gums that form gels in water. A well-known gum resin is myrrh, an extract from gum myrrh plants of the family Burseraceae, such as *Commiphora habessinica* (syn. *C. abyssinica*).

Balsams are physiological or pathological substances secreted by some plant species. They contain essential oils and resins and esters of organic acids (mainly benzoic and cinnamic acids). The well-known Peruvian balsam flows from injured trees of the genus *Myroxylon* (Fabaceae), such as *M. balsamum* var. *genuinum* and *M. b.* var. *pereirae*, which are native to Central and South America.

8.2.14.4 Other materials

Extraction is not only used for obtaining essential oils and oleoresins, but also other materials for flavouring foods, such as **extracts** and **tinctures** that are also acquired by extraction. Extraction with a solvent (mostly ethanol) by standing at room temperature is called maceration, and the product is the **macerate**. Extraction with the flow of a solvent that passes through a material is percolation, and the product thus obtained is the **percolate**. Products obtained by cold **maceration** or **percolation** usually have better organoleptic properties. Extraction at higher temperatures is often called **digestion**. An alcoholic (or aqueous) extract (or extract obtained using other solvents) is usually called a **tincture**. In practice, the basic extraction procedures are variously combined and modified. For example, hop extract is produced by two-stage extraction. The first stage is percolation of a volatile solvent (such as dichloromethane) to obtain the oil, which is followed by hot water extraction (extraction of tannins and so-called soft resins). Both extracts, after evaporation of solvents, are combined and homogenised. The name **essence** is used for alcoholic extracts of materials, but today it is used more for special composed products used for food flavouring that are mainly known as **aroma compositions**.

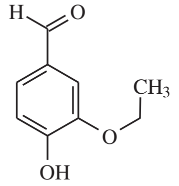
Other raw materials for producing food flavourings include **musts** or **juices** and **pulps** from fruits and vegetables. Citrus fruit juices, apple, grape, currant, cherry juices and other ingredients are all commonly used as the starting material. Concentrated apple juice is used as the basis for the production of other flavours (such as apricot); intensely coloured juices (e.g. juices from elderberries and blackcurrants) are used to dye various products. Vegetable juices that are usually used as basic raw materials for food flavourings include carrot, cabbage, tomato and onion and garlic juices. Fruit

and vegetable pulps are also used as a supporting medium for the production of paste-like flavours. The raw materials for flavour production also include various **distillates** (fruit and herbal), which usually contain ethanol.

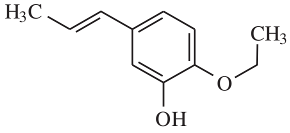
8.2.14.5 Synthetic compounds

Hundreds of different essential oils are used in current industrial practice. Their scarcity, higher price and differences in their quality have led to the production of cheaper **reconstituted essential oils**, which contain the same fragrances as natural essential oils, but are synthetic. These essential oil reconstitutions usually lack the subtle characteristics of natural essential oils.

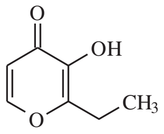
Examples of synthetic substances that do not occur in nature and yet find a use in food flavouring are ethyl vanillin, known as bourbonal (**8-211**), and (*E*)-2-ethoxy-5-(prop-1-en-1-yl)phenol (propenyl guethol, **8-212**) which has a vanilla-like smell. The advantage is that the smell of ethyl vanillin is between 2 and 4 times more intense than the smell of vanillin. Another commonly used compound is ethylmaltol (**8-213**), which has a caramel odour 4–6 times more intense than maltol. Allyl phenoxy acetate (**8-214**) has a sweet smell of honey and pineapple. One of the first synthetic substances with a strawberry flavour, used for confectionery, ice cream and other products, was (*E*)-isomer of ethyl 3-methyl-3-phenylglycidylic acid (ethyl 3-methyl-3-phenyloxiran-2-carboxylate, also known as fraseol or strawberry aldehyde, **8-215**). Only the (2*R*,3*R*)-isomer, however, has a desirable strawberry flavour. Ethyl 3-phenylglycidylic acid (ethyl 3-phenyloxiran-2-carboxylate, also known as raspberry aldehyde, **8-216**) found use as a flavouring agent in similar products that require the aroma of raspberries.



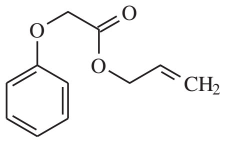
8-211, bourbonal



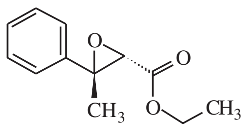
8-212, (*E*)-prop-2-en-1-ylguethol



8-213, ethylmaltol

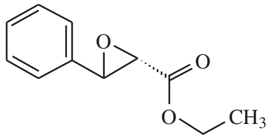


8-214, allyl phenoxyacetate



8-215, ethyl 3-methyl-3-phenyloxirane-2-carboxylate

Adulterating and diluting essential oils to increase profits can sometimes happen in the essential oil and aromatherapy industries.



8-216, ethyl 3-phenyloxirane-2-carboxylate

Adulterating an essential oil is relatively easy; forms of adulteration may include the addition of alcohol, synthetic products, substituting different and cheaper oils and passing them off as natural oils.

8.3 Taste-active substances

Oral ingestion of food and other substances in humans and higher animals are inseparable from the subjective taste sensations. The stimulae for irritation of taste receptors localised in the mouth, especially on the tongue, are **taste-active substances**. They are usually polar, water-soluble and non-volatile compounds. The resulting sensation is usually a combination of fundamental and other tastes:

- **sweet**
- **salty**
- **acid**
- **bitter**.

These basic taste sensations arise at relatively specialised receptors located in different places of the mouth. Sweet substances are perceived primarily at the tip of the tongue, salty substances in defined areas of the upper surface of the tongue, sour substances to the sides and bitter substances in the root of the tongue and soft palate. Taste receptors also respond to other stimuli (tastes) that are incorporated virtually in the whole oral cavity:

- **umami** (now considered to be a fifth basic taste)
- **astringent**
- **pungent** (burning or hot)
- **cooling** and others.

The interpretation of taste in terms of molecular interactions of taste-active compounds with biopolymers in taste receptors is probably analogous to the general scheme of pairs of interactions of the type of enzyme–substrate, hormone–receptor or antigen–antibody. These interactions and relationships between the chemical structure of a substance and its taste are best known (relatively) for sweet substances, but information on other substances is still largely incomplete.

The measure of taste intensity is the lowest detectable concentration of a substance in the solution causing the sensation, called the **threshold value**. As in the case of odour-active substances, **taste detection threshold values** and **taste recognition threshold values** are likewise recognised. Both values are measures of taste intensity.

8.3.1 Sweet substances

Sweet taste is commonly associated with sugars, especially sucrose (saccharose). Sweet substances are, with few exceptions, monosaccharides, oligosaccharides and sugar alcohols, but most of these are less sweet than sucrose. Many sugars are not even sweet at all and some, such as β-D-mannose and some oligosaccharides, are even bitter. In contrast, there are other compounds that have a completely different structure than sugars and yet are much sweeter than sucrose (e.g. synthetic sweeteners).

8.3.1.1 Sweet taste quality and intensity

Sugar and all sweet substances differ in their:

- sweet taste quality
- sweet taste intensity.

Sucrose has a particularly full taste, which is acceptable even at high concentrations. It is therefore used as a standard for sweet taste in the sensory evaluation of sweet substances. The threshold values of some sugars in aqueous solutions are listed in Table 8.37. For practical reasons, the sweetness of substances is expressed as a multiple of the sweetness of the sucrose solution (mainly 10% solution). The relative sweetness of certain sugars and sugar alcohols is given in Table 8.38. These values are only approximate, as they depend on sugar concentration, type and amount of anomers, temperature, presence of other substances and other factors.

For example, D-fructose solutions are sweeter than sucrose solutions, but in pastries and hot coffee both sugars show the same sweetness. The sweetest form is β-D-fructopyranose, which has about 180% of sucrose sweetness, but as a result of mutarotation, the sweetness of solutions decreases to about 150% of the sucrose sweetness because the individual anomers each have a different sweetness. The sweetness of fructose syrups depends on the fructose content. Syrups containing 42% fructose are about as sweet as sucrose solutions, syrups with 55% fructose show 100–110% of sucrose sweetness, and syrups with 90% fructose have 120–160% of sucrose sweetness.

Table 8.37 Taste threshold values of selected sugars.

	Detection threshold		Recognition threshold	
Sugar	Mol/l	%	Mole/l	%
D-Glucose	0.065	1.17	0.090	1.63
D-Fructose	0.020	0.24	0.052	0.94
Maltose	0.038	1.36	0.080	2.89
Saccharose	0.011	0.36	0.024	0.81
Lactose	0.072	2.60	0.116	4.19

Table 8.38 Relative sweetness of selected sugars and sugar alcohols (10% saccharose solution = 1).

Compound	Sweetness	Compound	Sweetness
Monosaccharides		***Trisaccharides***	
D-Xylose	0.70	Raffinose	0.15–0.20
D-Glucose	0.40–0.80	***Alditols***	
D-Mannose	0.30–0.60	L-Arabinitol	1.00
D-Galactose	0.30–0.60	Xylitol	0.90–1.20
D-Fruktose	0.90–1.80	D-Glucitol	0.40–0.60
Disaccharides		D-Mannitol	0.50–0.70
Invert sugar	0.95–1.80	Galactitol	0.40
L-Rhamnose	0.30	Maltitol	0.70–0.90
α,α-Trehalose	0.60	Isomaltitol	0.40–0.50
Maltose	0.30–0.60	Palatinitol	0.45
Lactose	0.20–0.60	Lactitol	0.30–0.40
Lactulose	0.60	***Various***	
Saccharose	1.00	Glycerol	0.50

In 10% solutions, the relative sweetness of D-glucose is about 50–60% of sucrose sweetness, while in solutions containing 50–60% glucose its sweetness is higher (90–100% of sucrose sweetness). β-D-Glucopyranose has about 66% of the sweetness of α-D-glucopyranose. The sweetness of glucose syrups depends on the glucose content and its lower oligomers. A syrup with a DE value of 30 has about 30–35%, syrup with a DE value of 36 has 35–40%, syrup with a DE value of 42 has 45–50%, syrup with a DE value of 54 has 50–55% and syrup with a DE value of 62 has 60–70% of sucrose sweetness.

Besides the sweet taste, some sweet substances show other side-tastes. For example, maltose and D-glucitol have the flavour of syrups and the taste of D-fructose solutions is fruity and slightly sour. Sucrose taste in threshold concentrations is even described as slightly bitter. Xylitol shows a cooling effect on dissolution.

Sweet substances are classified depending on various criteria:

- according to the origin as natural, synthetic identical to the natural or modified natural and synthetic substances (absent in nature);
- from the nutritional point of view to substances that are a source of energy and substances that have no nutritional value;
- medically contraindicated substances in diabetics (people with *diabetes mellitus*) and substances that do not increase blood

glucose levels; also recognised are cariogenic (causing tooth decay) and non-cariogenic sweet substances.

The relative sweetness of some non-sugar sweet natural, modified natural and synthetic compounds and other aspects of sweet substances, which include food additives (sweeteners), are described in Section 11.3.2.

8.3.1.2 Physiology, nutrition and use

Sucrose has major significance as a natural sweet substance, as do starch syrups (mixtures of D-glucose, maltose and maltooligosaccharides), D-glucose, invert sugar (equimolar mixture of D-glucose and D-fructose), D-fructose and lactose. Sugar alcohols, D-glucitol (also known as D-sorbitol), D-mannitol and xylitol, have found widespread use as sweeteners for diabetics. Other sugars, sugar alcohols and non-sugar natural sweeteners are, with a few exceptions, primarily the subject of academic rather than industrial interest. All these sweet substances have some nutritional value, while alcoholic sugars are non-cariogenic or slightly cariogenic substances. Modified natural and synthetic sweet substances used as sweeteners have, with some exceptions, no nutritional value, and are also non-cariogenic.

8.3.2 Salty substances

Salty tastes are exhibited almost exclusively by some inorganic salts (especially halides, sulfates, phosphates, nitrates and carbonates of alkali metals, alkaline earth metals and ammonium salts). Salty tastes combined with other tastes are also shown by some salts of carboxylic acids (salts of formic, acetic, succinic, adipic, fumaric, lactic, tartaric and citric acids), amino acids (such as salts of glutamic acid and choline) and some oligopeptides.

8.3.2.1 Salty taste quality and intensity

The quality of salty taste varies in different substances depending on the type of compound, its concentration and the presence of other ingredients. In the case of inorganic salts, both types of ions, cations and anions, are involved in the salty taste perception. For example, sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) differ in the intensity of salty taste (effect of anion), while sodium chloride (NaCl) and potassium chloride (KCl) do not have the same salty taste quality (the effect of cation). The intensity of the bitter taste usually increases with increased relative molecular weight (apparent diameters of the two ions) of salt. Only sodium chloride has a pure salty taste (the sum of the apparent diameters of the two ions is 0.556 nm), corresponding to the hydrated sodium cation in combination with the hydrated chloride anion released from the crystal lattice of NaCl. Other salty substances also exhibit more or less intense bitter taste or salty taste when combined with some other tastes, such as the metallic taste. For example, potassium bromide (KBr, 0.658 nm) tastes salty and bitter and potassium iodide (KI, 0.706 nm) is bitter as is magnesium chloride ($MgCl_2$, 0.850 nm). The character of salinity in many cases varies with salt concentration. Sodium chloride, which is considered the standard of salty taste, has a sweet taste at very low concentrations. Potassium chloride at low concentrations has a sweet taste that is enhanced with increasing concentration, while the intensity of the bitter taste also increases. At higher concentrations it tastes bitter and salty with a slightly sour taste.

The quality of the salty taste of foods depends on the ratio of sodium cations (Na^+) and chloride anions (Cl^-). Foods with natural levels of these ions, however, do not always taste salty, because both ions may be not present in the required stoichiometric ratio. The quality of salty taste of a mixture of salty substances depends on their type and mutual ratio, which is employed to compose the salt substitutes used in various diets. The intensity of the salty taste depends on the concentration of salty substances and the presence of other components in the mixture. The salinity of various salts is an additive property, but some mixtures exhibit synergism, which means that the intensity of a mixture of salty compounds is higher than the sum of salty tastes of its components. The threshold concentrations of the most common salty substances are listed in Table 8.39.

In practice, the most important salty compound is sodium chloride, which occurs in lower or higher quantities in virtually all foods as a natural constituent or an intentionally added additive. The content of NaCl in selected foods is given in Table 8.40. According to the sodium content (salt contains 38.4% sodium), which roughly corresponds to the NaCl content, foods can be divided into four categories:

- foods with very low Na content (usually containing a thousandth of a gram, but less than 0.4 g of sodium per 1 kg food), such as fruits, fresh vegetables, most fats, sugar, confectionery and some dairy products;
- foods with low Na content (0.4–1.2 g/kg), such as fresh meat, fish, poultry, milk and dairy products (except hard and processed cheeses) and some edible fats;
- foods with high Na content (1.2–4.0 g/kg), examples are some types of bread and pickles;
- foods with very high Na content (more than 4.0 g/kg), such as smoked meat products, hard and processed cheeses, some bakery

Table 8.39 Detection threshold concentrations of selected salty inorganic salts.

Salt		Threshold concentration (mg/l)
Sodium chloride	NaCl	160–240, 584, 296–839,[a] 1750
Potassium chloride	KCl	1267, 345–1270[b]
Ammonium chloride	NH_4Cl	214
Magnesium chloride	$MgCl_2$	1430

[a] In men, higher by about 50% compared with women.
[b] In men, higher by about 40% compared with women.

Table 8.40 Sodium chloride content in edible portions of selected foods.

Food	NaCl (g/kg)	Food	NaCl (g/kg)
Meat (beef, pork, poultry)	1.82–2.02	Rice	0.15
Meat products	12–50	Lens	0.15
Fish	0.98–5.50	Fresh fruits	0.04–0.08
Milk	0.88–2.25	Fresh vegetables	0.05–1.55
Dairy products	0.83–80	Canned vegetables	5.0–24.5
Butter	1.30	Potato	0.48
Eggs	1.50	Beer	0.13
Flour	0.050	Wine	0.05–0.08
Bread and pastries	0.55–10.4	Nuts	0.05–0.75

products, dried soups, olives and vegetables in brine, salty snacks and similar products.

Poultry products usually have a lower NaCl content than pork meat products; the highest content of NaCl is found in uncooked and raw smoked meat, dry or hard salami, some cheeses and fish. For example, the dry pork salami NaCl content may be 58 g/kg and beef jerky contains 50 g/kg NaCl. Salted fish contain an extremely high salt content (dry and salted cod contains 180 g/kg Na) so before eating it is necessary to remove NaCl by digesting in water. With regard to dairy products, the highest content of NaCl is found in cheeses (parmesan 40 g/kg, blue cheese 36 g/kg, feta 28 g/kg, camembert 21 g/kg and cheddar 16 g/kg). Egg white and yolk contain NaCl as a natural component at levels of 0.25 and 12.5 g/kg, respectively. Examples of fruit products with extremely high salt content are olives pickled in salty brine. Fermented olives have an NaCl content of 60–90 g/kg, black unfermented olives only 10–30 g/kg. Various paste type soup products have even higher sodium chloride content, such as broths (about 650 g/kg), soy sauces (about 180 g/kg) and acid protein hydrolysates used as soup seasonings (200 g/kg).

Sodium added to food is not always in the form of NaCl. Common food additives, such as baking soda, some preservatives and monosodium glutamate also contribute to the total amount of sodium we consume (see Section 6.3.1).

8.3.2.2 Physiology and nutrition

Salty substances exhibit a variety of pharmacological effects, whose character depends on the type of cation and anion. Some substances are toxic at higher concentrations. The compound consumed in the largest amount is sodium chloride. The daily intake of salt in developed countries is estimated at 8–15 g. Sodium chloride supports the perception of taste of foods at the required intensity and fullness, stimulates not only receptors for salty taste, but significantly increases the perception of the sweet taste of sucrose and some other sweet substances, as well as sour taste perception, and suppresses the sensation of metallic taste and some other off-tastes, feel of diluted (watery) taste, optimises the resulting taste sensation and promotes balance between various basic tastes.

Sodium chloride is essential for the human body. Excessive intake of NaCl, however, causes fluid retention, swelling, burdens the kidneys, heart and blood circulation, and contributes to the emergence of hypertension. In industrialised countries, the consumption of NaCl in the majority of the population is excessive. It is recommended that it should be reduced below 10 g per person per day (see Section 6.3.1). Some diseases (hypertension, renal insufficiency and oedema) need a diet with a limited supply of salt or a completely salt-free diet. In these cases, the palatability of food is achieved by recipe modification, especially by the addition of spices or salt substitutes, in which the Na^+ cation is replaced by other cations, especially by the K^+ cation.

8.3.2.3 Use

Sodium chloride is added to food for the following reasons:

- to achieve the desired organoleptic properties of food products and dishes;
- to improve the processing conditions (e.g. salt in the manufacture of bread strengthens gluten in the dough and thus contributes to the stability of dough during mechanical processing, in the manufacture of processed cheeses NaCl, which is part of the cheese-melting salts, displaces calcium from milk proteins, in the meat industry NaCl increases the meat binding capacity, in sausages it increases protein solubility and emulsification of coagulated proteins with fat and water to the desired structure);
- to achieve conservation, which lies in the ability of NaCl to reduce water activity below the level required by the growth of undesirable microorganisms, which is known as the bacteriostatic effect;
- to control desirable fermentation processes by suppression of growth of undesirable microflora (e.g. during dough rising,

cheese ripening, lactic acid fermentation of olives, cucumbers and cabbage).

Other saline substances have found application almost exclusively in salt substitutes, which are used in diets with reduced salt content (low sodium diets) for patients who have to radically reduce sodium intake. A salt-free diet is suitable in cases of failure of the excretion of sodium ions and water retention in the body, which results in massive swelling, especially of the feet (occurring during certain heart and kidney diseases or during pregnancy). The main salt substitute is potassium chloride, the properties of which are similar to sodium chloride properties. This suits quite well in terms of technological and preservative properties, but less so in terms of taste, because it has a strong bitter taste in addition to its salty taste. Potassium chloride is therefore used in mixtures with other salty compounds to correct its taste. The cations Mg^{2+}, Ca^{2+} and $NH_4{}^+$ are also used in salt substitutes in various combinations with inorganic and organic anions (chlorides, sulfates, phosphates, citrates, succinates, lactates, acetates and formates). Some dipeptides also have a salty taste (see Section 2.3.3.2) and monosodium glutamate is often used as a salty taste enhancer. Compounds used as substitutes for table salt are often very complex and are subject to a number of patents.

8.3.3 Sour substances

The acidity of food is related to the amount of undissociated and dissociated forms of carboxylic acids and oxonium ions, respectively, that are present. The major substances that give a sour taste in foods are undissociated hydroxycarboxylic acids, citric and malic acids. Often, however, other carboxylic acids occur, such as ascorbic acid in most types of fruits, tartaric acid in grapes, isocitric acid in blackberries, oxalic acid in rhubarb, lactic acid in some dairy products (such as yoghurt), fermented cucumbers, cabbage, olives, vinegar and propionic acid in Emmental type cheeses (see Section 8.2.6). The acidity of cola drinks is provided by phosphoric acid, sometimes accompanied by citric or other acids. Carboxylic acids also act as bacteriostatic agents and affect a number of biochemical and chemical reactions.

8.3.3.1 Sour taste quality and intensity

Individual acids differ in the nature of their sour taste, and often in the quality. Threshold concentrations of sour taste perception of different acids also differ to a certain extent. They vary over a rather wide range depending on a number of subjective and objective factors, such as sensory analysis methodology, quality of respondents, quality of water and its pH (Table 8.41).

Less important for the perception of sour taste are hydrogen ions (H^+), and correspondingly oxonium cations (H_3O^+) resulting from the dissociation of acids:

$$R\text{–}COOH + H_2O \rightarrow R\text{–}COO^- + H_3O^+$$

The number of oxonium ions, related to pH of the biological systems, is an important criterion as it affects the redox potential of the system, ongoing enzymatic and chemical reactions, growth of microorganisms and likewise the odour, taste and colour of foods. The pH value depends on the concentration of the acids, their dissociation constants and the degree of neutralisation of the acids by basic components. For various reasons (microbiological, technological and others), it is useful to divide foods into:

- very acidic (pH is <4.0)
- less acidic (pH ranges from 4.0 to 6.5)
- non-acidic (pH > 6.5).

Table 8.41 Detection thresholds of selected acids in water according to various workers.

Acid	Detection threshold (mg/l)	Acid	Detection threshold (mg/l)
Acetic	19.9,[a] 54.1,[b] 110, 175[c]	Succinic	556
Lactic	15.1,[a] 133,[b] 200	Malic	110
Pyruvic	22.6,[a] 47.2[b]	Tartaric	80
Oxalic	18.6[a]	Citric	26.4,[a] 100–130, 150, 350[b]

[a]In water, pH 4.3.
[b]In water, pH 6–7.
[c]In beer.

Fruit is, with few exceptions, always very acidic and the maximum acid levels in fruits occur in the period before full maturity. The pH of fruit juices is generally lower than 4.0, but in over-ripe pears, cherries and peaches may increase up to pH 4.5 and in over-ripe elderberries may even reach a value of 4.7. The level of acids in the fruit depends on the type, and is usually 10–30 g/kg, a lower amount of acids (about 1 g/kg) is found in pears, while citrus fruits contain higher amounts of acids (about 80 g/kg). A sour taste is often modified by the presence of carbohydrates, tannins, ethanol or various cations and other substances. Carbohydrates weaken the sour taste of acids and tannins, and alcohol, on the contrary, emphasises their taste.

Fresh vegetables are, in comparison with fruits, relatively poor in acids. The pH values of common vegetables vary, usually from 5.0 to 6.6. An exception is rhubarb, where the pH is around 3.2; tomatoes are also quite acidic with a pH of about 4.3. The content of acids in fresh vegetables usually ranges between 2 and 4 g/kg.

Meat and other animal products are even less acidic (pH = 6.6–7.2) than fresh vegetables. Immediately after slaughter, glycogen in muscles is broken down and the acidity of meat increases somewhat due to a temporary increase in lactic acid content (pH of about 5.8, see Section 2.4.5.1.5), but never reaches the acidity of very acidic food (pH < 4.0).

Fresh milk is a non-acid food (pH varies in the narrow range of from 6.50 to 6.75), while the pH of yoghurt and other fermented dairy products ranges from 4.0 to 4.2. The pH value of a typical hard cheeses is about 5.1.

Chicken egg white is completely non-acidic, but during storage the pH increases from the original value of 7.5 in fresh eggs up to 9.0 or higher. Egg yolk is more acidic, as its pH is around 6.2.

8.3.3.2 Physiology, nutrition and use

Organic acids, hydrogen salts, some mineral acids or hydroxy-carboxylic acid lactones have found use as acidifying agents and acidulants for modifying pH, but they are also used for other purposes (such as antimicrobial agents). Addition of some salts or alkaline agents, on the contrary, reduces the acidity of certain foods (such as acidity of cocoa beans during roasting). The use and medical evaluation of these compounds is described in Section 11.2.4.

8.3.4 Umami and kokumi tastes

The term umami is relatively new in the West, but it is not new to the Japanese, who have used this term since the early 1900s to describe the delicious, full-bodied, savoury or meaty taste bringing a sense of satisfaction and increased viscosity in the mouth, which is translated from Japanese as savoury or yummy. Being able to distinguish the umami taste takes some practice, because it is not as obvious as other tastes, such as sweet or bitter. Umami taste is caused mainly by glutamic acid and its corresponding salt, sodium hydrogen glutamate, which is the predominant form of glutamic acid at pH values ranging from 4.3 to 9.5. The taste intensity of glutamate is quite strong. The detection threshold is 120–170 mg/l with a recognition threshold of 300 mg/l. The word corresponding to umami in Chinese is *xian wei*.

Umami, spicy and meaty tastes are also found in a number of other substances, for example in glycoconjugates of glutamic acid produced in the Maillard reaction, such as *N*-glycoside, *N*-(D-glucose-1-yl)-L-glutamic acid, and the corresponding Amadori compound, *N*-(1-deoxy-D-fructose-1-yl)-L-glutamic acid. Umami taste is also shown by peptides such as Glu-Glu-Leu and lactoyl-Glu, which have been detected in Parmesan cheese, β-alanyl dipeptides found in bouillons (see Section 2.3.3.1.3), *N*-(1-carboxyethyl)-6-hydroxymethylpyridinium-3-ol inner salt (alapyridaine), identified in heated sugar/amino acid mixtures and in beef bouillon, morelid, a malic acid glucopyranoside from morel mushroom and (*R*)-2-(carboxymethylamino)propanoic acid, known as (*R*)-strombine, isolated from dried scallops (**8-217**). Some other amino acids and peptides also show umami taste, but have not found practical applications. An example is ibotenic acid (naturally occurring in fly agaric *Amanita muscaria* and panther cap *A. pantherina*, Amanitaceae) that has umami taste 5–30 times more intense than glutamate. Anomers of (*S*)-malic acid 1-*O*-D-glucopyranoside known as (*S*)-morelid (**8-217**) contribute to the umami taste of morel mushrooms (*Morchella deliciosa*, Morchellaceae). Threshold concentrations for the umami-like, slightly sour taste of morelid is 1.9 g/l.

In the past, sodium hydrogen glutamate was linked with the so-called Chinese restaurant syndrome, which is manifested by fairly typical and unspecific sensations, such as headache, flushing, sweating, facial pressure or tightness, numbness, tingling or burning in the face, neck and other areas, chest pain and nausea. Latterly, sodium hydrogen glutamate has been classified as a food ingredient that is generally recognised as safe, but its use as a flavour enhancer remains controversial. For this reason, when this compound is added to foods, legislation requires that it be listed on the label.

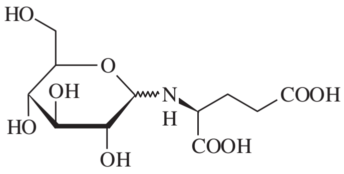
Kokumi (different from umami) is another food attribute identified by the Japanese. It is sometimes translated as heartiness or mouthfulness and describes compounds in food that do not have their own flavour or have a distinct flavour, but enhance the flavours with which they are combined by triggering calcium receptors in the tongue. In foods system, there are three types of flavour sensations attributed to kokumi:

- mouthfulness and continuity (long lasting taste development)
- punch (initial taste and impact)
- mildness (roundness and balance).

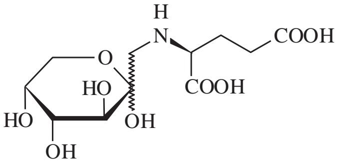
Kokumi compounds supposedly include calcium, protamines (found, for example, in fish sperm), histidine, glutathione and other peptides, such as γ-glutamyl peptides or the peptides of mature Gouda cheese. Another example of a mouthfulness (kokumi) enhancing molecule is the bitter hydrophobic compound in thermally processed avocado fruits, (12*Z*,15*Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene (see **8-225**, later).

A special group of taste-active compounds are umami and kokumi enhancers used as food additives. Examples of these substances are 5′-nucleotides, such as inosine 5′-monophosphate (5′-IMP, inosinic acid disodium salt) and guanosine 5′-monophosphate (5′-GMP), which is about twice as active in enhancement of the umami taste of glutamate solutions, while xanthosine 5′-monophosphate (5′-XMP) and adenosine 5′-monophosphate (5′-AMP) are less active, showing only about 0.5 and 0.1 times the impact of 5′-IMP (see Section 11.3.5). Umami enhancing molecules are also particular derivatives of 5′-nucleotides arising in the Maillard reaction of 5′-GMP. An example is lactamide N^2-lactoylguanosine 5′-monophosphate found in dried bonito. It is formed in the reaction of 5′-GMP with lactic acid. Another example is (*R*)- and (*S*)-N^2-(1-carboxyethyl)guanosine 5′-monophosphate (**8-217**), which was isolated from yeast extract. It is formed from 5′-GMP and dihydroxyacetone (glyceraldehyde). Active molecules also include many other Maillard reaction products of 5′-GMP and various synthetic derivatives of 5′-nucleotides.

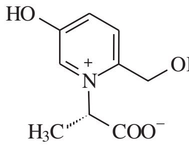
Another group of taste enhancers arises from bitter tasting creatinine and reducing hexoses in the Maillard reaction. The identified compounds include, *N*-(1-methyl-4-oxoimidazolidin-2-ylidene)aminoacetic acid derived from glycine, *N*-(1-methyl-4-oxoimidazolidin-2-ylidene)aminopropionic acid derived from alanine and *N*-(1-methyl-4-oxoimidazolidin-2-ylidene)amino-4,5,6-trihydroxyhexanoic acid (**8-217**) derived from glucometasaccharinic acids, such as 3-deoxy-D-gluconic acid (Figure 4.48). At sub-threshold concentrations these substances and also some other synthesised *N*-(1-methyl-4-oxoimidazolidin-2-ylidene)-α-amino acids enhanced the typical thick-sour and mouth-drying sensation and the mouthfulness imparted by stewed beef juice.



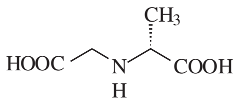
N-(D-glucose-1-yl)-L-glutamic acid



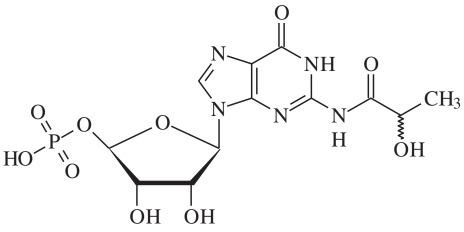
N-(1-deoxy-D-fructose-1-yl)-L-glutamic acid



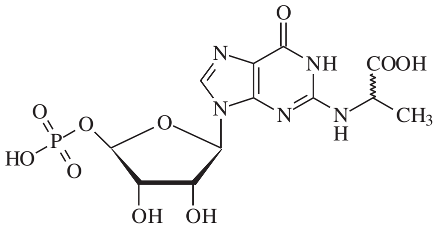
(+)-(*S*)-alapyridaine



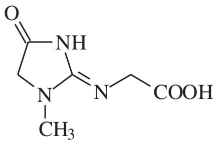
(*R*)-strombine



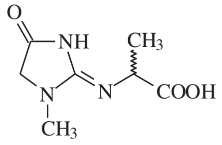
N^2-lactoylguanosine 5′-monophosphate



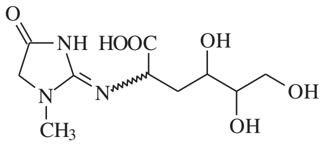
N^2-(1-carboxyethyl)guanosine 5′-monophosphate



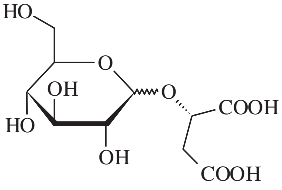
N-(1-methyl-4-oxoimidazolidin-2-ylidene) aminoacetic acid



N-(1-methyl-4-oxoimidazolidin-2-ylidene)-aminopropionic acid



N-(1-methyl-4-oxoimidazolidin-2-ylidene)amino-4,5,6-trihydroxyhexanoic acid



(*S*)-morelid

8-217, compounds with umami taste and taste enhancers

8.3.5 Bitter substances

Bitter substances of foods are usually classified according to the origin into compounds that:

- are characteristic natural components of certain foods and their occurrence is genetically determined;
- are formed during processing and storage of foods by chemical reactions or activities of their own enzyme systems;
- result from contamination by certain organisms growing on food materials;
- have been intentionally added to food as additives.

A number of organic compounds commonly present in foods have a bitter taste, such as certain fatty acids, amino acids, peptides, amines, amides, ketones, nitrogen-containing heterocyclic compounds (including alkaloids) and many other compounds. Their bitter taste in food is usually seen at higher concentrations. Certain inorganic salts are also bitter.

A bitter taste is desirable for some foods, where it is a typical taste. Examples include grapefruits, chicory leaves, cocoa, coffee, beer and tonic drinks. However, sometimes a bitter taste is considered undesirable (off-flavour) and the affected foods can have an unacceptable taste and can be even inedible (such as oranges, carrots

Table 8.42 Detection threshold concentrations of selected bitter compounds according to various workers.

Compound type	Bitter compound	Detection threshold (mmol/l)	Compound type	Bitter compound	Detection threshold (mmol/l)
Inorganic salts	Magnesium sulfate	5	Cucurbitacins	Cucurbitacin C	0.000 2
Amides	Propionamide	52	Floroglucinols	Isohumulone	0.02
	Propylamine	20	Flavonoids	(+)-Catechin	2
	Butylamine	6		Naringin	0.2 (0.04)
	Diethylamine	3		Procyanidin B_3	0.07
Amino acids	L-Tryptofane	4	*N*-Heterocycles	Pyridine	2
	L-Leucine	15		Pyrazine	13
Fatty acids	Oleic acid	9–12		Methylpyrazine	7
	Elaidic acid	22		Acetylpyrazine	1
	Linoleic acid	4–6		Imidazole	6
	α-Linolenic acid	0,6–1,2	Limonoids	Limonin	0.08 (0.003)
	γ-Linolenic acid	3–6	Phloroglucinols	Iso-α-acids	0.02
	Arachidonic acid	6–8		Tetrahydroiso-α-acids	0.007
Alkaloids	Quinine	0.03 (0.025, 0.01, 0.008)	Quassinoids	Quassin	0.0002
	Caffeine	2 (0.7, 0.02)	Secoiridoids	Amarogentin	0.000 03

and cottage cheese). Bitterness is related to the hydrophobicity of the molecules of the bitter compound, the size of the non-polar part of the molecule, its configuration and also the presence of at least one polar functional group is required. Data have been obtained for the results of sensory analysis under defined conditions, often referred to the bitter taste standard, which is usually quinine, or caffeine (which is about 60 times less bitter than quinine). Compounds with the threshold concentrations lower than 0.1 mol/l are generally considered to be very bitter. Table 8.42 gives some examples of threshold concentrations of selected bitter substances.

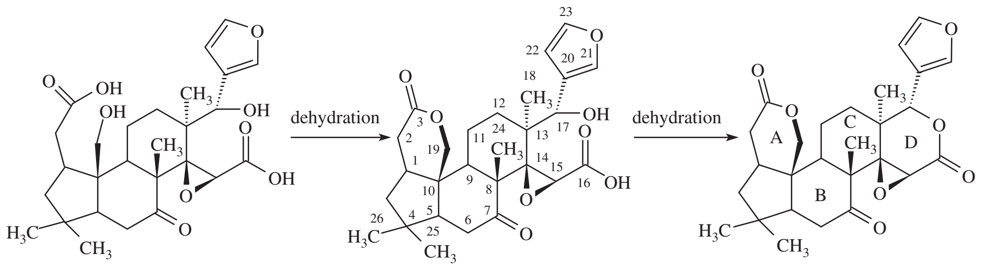
8.3.5.1 Bitter substances naturally present and formed during food processing and storage

8.3.5.1.1 Fruits

The typical natural bitter taste of certain citrus fruits and juices is caused by the presence of flavanone-7-glycosides, which have as a sugar component disaccharide neohesperidose and are therefore known as **neohesperidosides**. Aglycones are not bitter. The bitter component of grapefruits is naringin (naringenin-7-*O*-neohesperidoside). Some bitter odour-active compounds also contribute to the bitter taste of grapefruits, such as the sesquiterpenic ketone nootkatone and other bitter substances. The bitter constituent of bitter oranges also known as bigarade oranges (*Citrus aurantium* subsp. *amara*) is neohesperidine (hesperetin-7-*O*-neohesperidoside).

A bitter taste also often occurs in otherwise sweet oranges during juice processing and storage, due to the presence of metabolically altered (degraded), triterpenoids (tetranortriterpenoids) that are known as **limonoids**. The basic structure consists of four six-membered rings and one furan ring. These secondary metabolites occur as aglycones, glucosides or A-ring lactones. More than 300 limonoids are known, which are found in the rue (citrus) family Rutaceae, to which oranges also belong, in the mahogany family Meliaceae, in the family *Cneoraceae* and in plants of the genus *Harrisonia* of the family Simaroubaceae.

The most abundant aglycone and glucoside for most citrus species are limonin and limonin 17β-D-glucopyranoside with the sugar moiety bound to the C-17 hydroxyl group (the aglycone is called limonoic acid lactone or limonate A-ring lactone) and are biosynthesised by transformation (oxidation, isomerisation, rearrangement and elimination of four carbon atoms of the side chain) of 4,4-dimethylsterol (known as euphol) via butyrospermol and other metabolites, such as non-bitter limonoic acid and its lactone (ring A). The biosynthesis takes place in the leaves and limonoids are then transported to the fruits and seeds in particular. Limonin glucoside is stable in neutral media, but hydrolysis of the sugar moiety in acidic juices under the action of β-glucosidase and dehydration (to form another δ-lactone in ring D) yields intensely bitter limonin (Figure 8.90) and its epimer, which is formed through the inversion of the C-17 substituent. The formation of limonin is accelerated by juice pasteurisation. The bitter taste is reflected at a limonin content higher than 6 mg/l. Limonin can also be found in lemons and grapefruits.

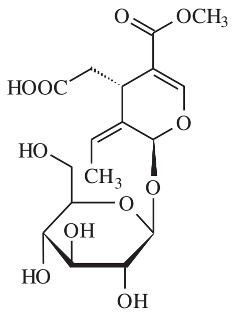


limonoic acid — limonoic acid lactone — limonin

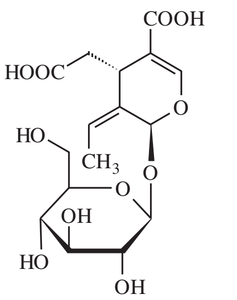
Figure 8.90 Formation of limonin from non-bitter precursor.

Several dozen limonin-related compounds (**8-218**) have been found in citrus fruits. All citrus limonoids contain a furan ring attached to ring D at C-17 and oxygen-containing functional groups in positions C-3, C-4, C-7, C-16 and C-17 and C-14,15 of the epoxy ring. In addition to limonin, only three other limonoids, namely nomilin, ichangin and nomilinoic acid, are bitter compounds. Analogously to limonin, nomilin, nomilinoic acid and other limonin-related compounds occur in citrus fruits as aglycones and 17β-D-glucopyranosides. Of these compounds, only nomilin (which is about twice as bitter as limonin, but occurs in smaller quantities) may be involved in the bitter taste in citrus juices, together with limonin. Limonoids are also reported to possess multiple health promoting properties.

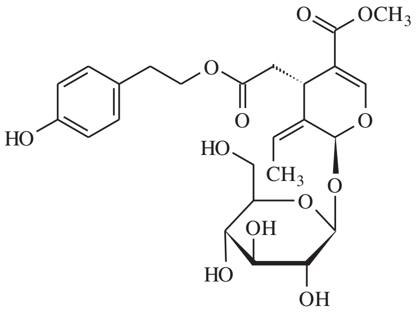
Olives, fruits of the olive tree (*Olea europea*) of the family (Oleaceae), have a markedly bitter taste, because they contain unusual **secoiridoids** (see Section 9.10), and their corresponding β-D-glucosides, known as **oleosides**, which protect the fruit against attack by microorganisms and insects. Small green olives contain the oleoside 11-methyl ester (elenolic acid β-D-glucoside formed by biosynthesis from 7-deoxyloganic acid, **8-219**), hydrolysis of which gives rise to the oleoside demethylelenolic acid β-D-glucoside (**8-220**). Elenolic acid β-D-glucoside is a precursor of ligstroside (**8-221**), which is an ester of tyrosol. A related compound, tyrosol ester oleocanthal (**8-222**), is found in extra virgin olive oil, where it is responsible for its slightly tangy, peppery bite in the back of the throat. Concentrations of oleacanthal, which shows anti-inflamatory properties, in extra virgin olive oils range from 22 to 190 mg/kg. None of the above compounds are bitter, however during fruit growth and maturation, ligstroside is oxidised (hydroxylated) to bitter oleuropein (**8-223**), a compound derived from 3,4-dihydroxyphenylethanol (3-hydroxytyrosol or hydroxytyrosol) and in olives that reach normal size, ligstroside does not occur. Oleuropein is found in green and yellow fruits, where its concentration can reach up to 140 mg/kg dry matter. Its content decreases during fruit ripening and is almost zero in the fully



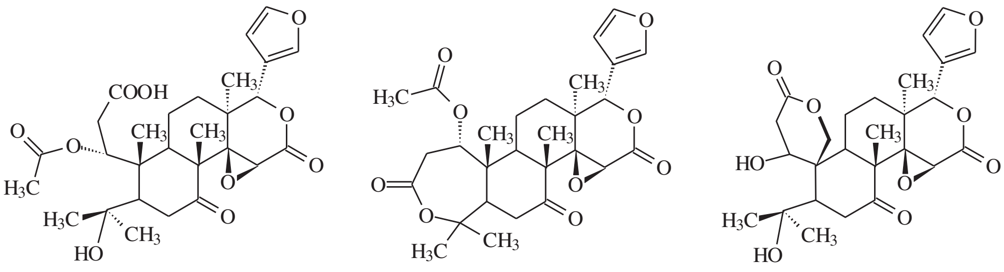
8-219, methyl oleoside



8-220, oleoside

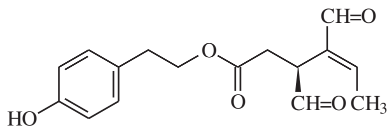


8-221, ligstroside



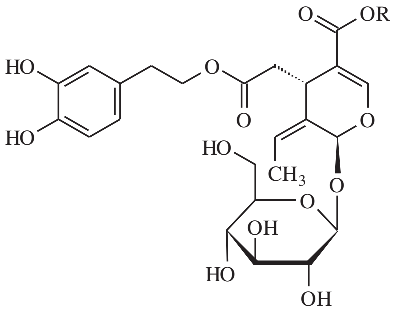
nomilinoic acid — nomilin — ichangin

8-218, minor bitter limonoids of oranges



8-222, oleocanthal

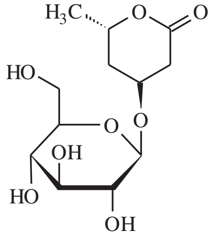
mature blue fruits. Ripe fruits contain only demethyleuropein (**8-223**) formed by hydrolysis of oleuropein. Hydrolysis by esterase yields hydroxytyrosol and elenolic acid β-D-glucoside, which is then hydrolysed by β-glucosidase to elenolic acid and glucose. Hydrolysis of oleuropein by β-glucosidase yields glucose and the corresponding aglycone that is hydrolysed by esterase to 3-hydroxytyrosol and elenolic acid. Small green olives also contain ligstroside, which is a derivative of tyrosol and elenolic acid methyl ester. Tyrosol and hydroxytyrosol also contribute to the bitter taste of olives. Hydroxytyrosol is present in olives likewise as 4-β-D-glucopyranoside. Its amount increases with olive ripeness.



8-223, oleuropein, R = CH_3
demethyloleuropein, R = H

Debittering of immature green olives is done either by lactic acid fermentation or hydrolysis of bitter glycosides by dipping in solutions of sodium hydroxide. Bitter oleuropein is hydrolysed in alkaline media to glucose and the corresponding aglycone, which yields 3-hydroxytyrosol (3,4-dihydroxyphenylethanol), methanol and demethylelenolic acid.

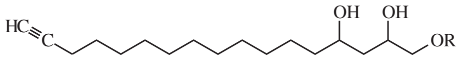
The bitter taste sometimes occurring in stewed sour cherries, plums and other stone fruits of the Rosaceae family is caused by the presence of cyanogenic glycosides, especially prunasin (see Section 10.3.2.3.1). Rowan berries, the fruit of rowan (*Sorbus aucuparia*), which are used to make jam or jelly with a distinctive bitter taste, contain 3-hydroxy-5-hexanolide β-D-glucoside (**8-224**). Enzymatic



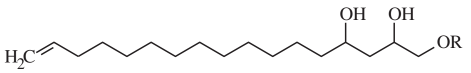
8-224, (3*S*,5*S*)-3-(β-D-glucopyranosyloxy)hexano-5-lactone

hydrolysis of this glycoside and dehydration of aglycone yields parasorbic acid, ring opening and further dehydration of parasorbic acid produces sorbic acid. Some selected rowan cultivars have less bitter or non-bitter fruits.

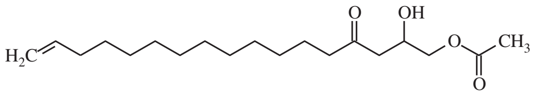
Raw avocado (*Persea americana*, Lauraceae) contains only trace levels of bitter compounds, but heat treatment (canning and preserving fruit) or air-drying induce the development of an unpleasant bitter after-taste. The substances responsible for the bitter taste are mainly various saturated and unsaturated C_{17}–C_{21} fatty acid derivatives with 1,2,4-trihydroxy- (triols), 1-acetoxy-2,4-dihydroxy- (acetates of triols) and 1-acetoxy-2-hydroxy-4-oxo groups (acetates of oxodiols). The most bitter compound is (12*Z*,15*Z*)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene, closely followed by other structurally related compounds. Examples of these molecules are given in formulae **8-225**. Natural constituents of avocado fruits of analogous structures are acetates of long-chain unsaturated fatty acids (see Section 3.3.3.2) and furans with long-chain saturated and unsaturated substituents (see Section 8.2.11.1.1).



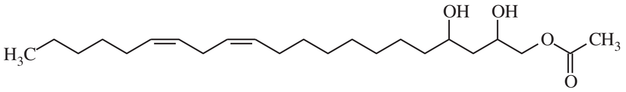
1,2,4-trihydroxyheptadeca-16-yne, R = H
1-acetoxy-2,4-dihydroxyheptadeca-16-yne, R = $COCH_3$



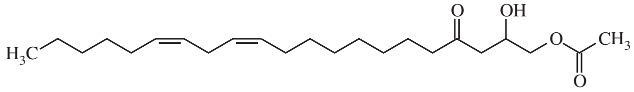
1,2,4-trihydroxyheptadeca-16-ene, R = H
1-acetoxy-2,4-dihydroxyheptadeca-16-ene, R = $COCH_3$



1-acetoxy-2-hydroxy-4-oxoheptadeca-16-ene



(12Z,15Z)-1-acetoxy-2,4-dihydroxyheneicosa-12,15-diene



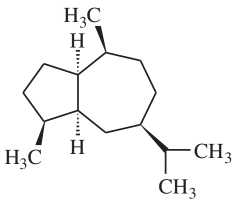
(12Z,15Z)-1-acetoxy-2-hydroxy-4-oxoheneicosa-12,15-diene

8-225, bitter compounds in avocado

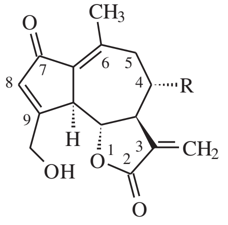
8.3.5.1.2 Vegetables

A number of vegetables from the family Asteraceae have a bitter taste, such as endive (*Cichorium endivia*), the aerial part (leaf rosette) of which is eaten as a salad. Lettuce (*Lactuca sativa*) sometimes has a bitter taste, particularly the stalk and the white milky juice. Chicory (*C. intybus* var. *foliosum*) is slightly bitter, and is cultivated for salad leaves called chicons (etiolated buds) growing from the root vertex in the dark. Roasted chicory root (*C. i.* var. *sativum*) is used in the manufacture of coffee surrogates. Bitter substances of these vegetables are primarily sesquiterpenic

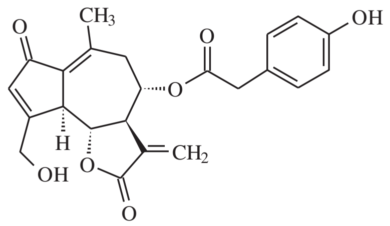
lactones derived from sesquiterpenic hydrocarbon guaiane, known under the systematic name of (1*S*,3a*S*,4*S*,7*R*,8a*S*)-7-isopropyl-1,4-dimethyldecahydroazulene (**8-226**). The main compound is lactucin (**8-227**), whose biosynthesis is based on sesquiterpenic hydrocarbon (+)-germacrene A. The highest concentration of bitter substances is present in the central axis of chicory buds that contain about 100–250 mg/kg, of which approximately 50% is lactucin, 30% 4-deoxylactucin (**8-227**) and 5% lactucopicrin (**8-228**). Their 11,13-dihydro derivatives are present in small amounts. The bitter taste of chicory buds also comes from coumarins and their glycosides (see Section 10.3.2.5.2), especially scopoletin, umbelliferone, aesculin (aesculetin 6-*O*-β-D-glucoside) and cichoriin (aesculetin 7-*O*-β-D-glucoside). The main bitter substance of the leaves of the artichoke thistle, known as cardoon (*Cynara cardunculus*), and of cultured varieties of globe artichoke (*C. c.* var. *scolymus*) of the same plant family, is cynaropicrin (**8-229**), which is also derived from guaiane. Cynaropicrin represents 60–80% of bitter substances of these plants. It is about twice as bitter as quinine and is sometimes used as a bittering substance in citrus drinks.



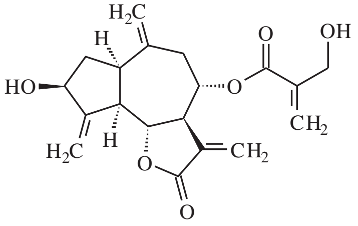
8-226, guaiane



8-227, lactucin, R = OH
4-deoxylactucin, R = H

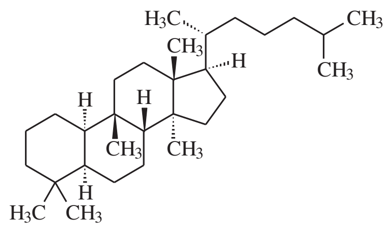


8-228, lactucopicrin

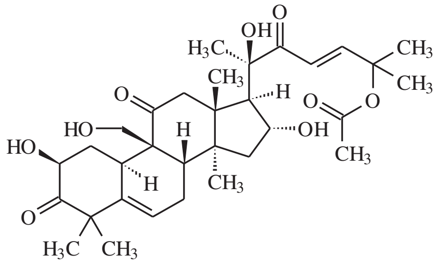


8-229, cynaropicrin

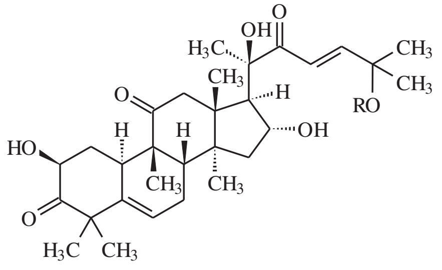
Vegetables of the cucurbit family (Cucurbitaceae), which includes squashes, melons, pumpkins, cucumbers and gourds of the genera *Benincasa*, *Citrullus*, *Cucumic*, *Cucurbita*, *Lagenaria* and *Luffa*, occasionally show bitter taste in response to various stress factors. The bearers of bitter taste are tetracyclic triterpenoids, called **cucurbitacins**, which are derived from the triterpenic hydrocarbon 5α-cucurbitane (**8-230**). Cucurbitacins are found in vegetables in levels of <0.01% as free compounds (aglycones) or as glycosides. Identified in the Cucurbitaceae family are cucurbitacins A–L (**8-231** to **8-238**), the most common of which are cucurbitacins C and D. The main bitter substance of cucumber is cucurbitacin C, pumpkins contain cucurbitacins E and B, sometimes cucurbitacins D and I, and cucurbitacin E is found in water melons. Cucumber seeds contain cucurbitacins A–I (**8-231** to **8-234**). Cucurbitacins and their glycosides are also found in plants belonging to several other families, such as Brassicaceae, Begoniaceae, Scrophulariaceae Primulaceae and Rosaceae. Their insecticidal, anti-tumour and anti-inflammatory effects are currently being studied. For example, under normal conditions cucumbers contain cucurbitacins throughout the plant (in the stem, leaves, cotyledonary leaves), but not in fruits. Various stress factors, such as significant difference between night and day temperatures, water logging or over-drying, however, manifest themselves with bitter fruits. The threshold levels of the main cucurbitacin C is about 1000 times lower than that of caffeine,



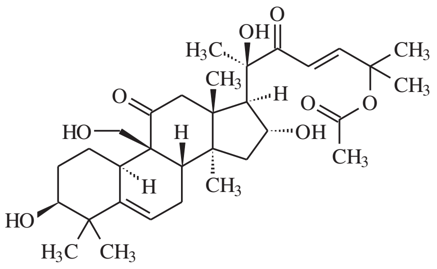
8-230, 5α-cucurbitane



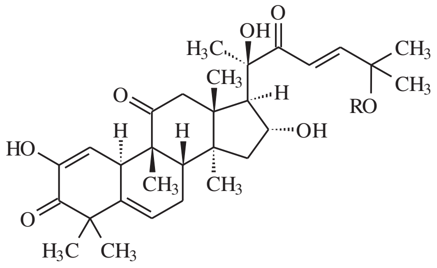
8-231, cucurbitacin A



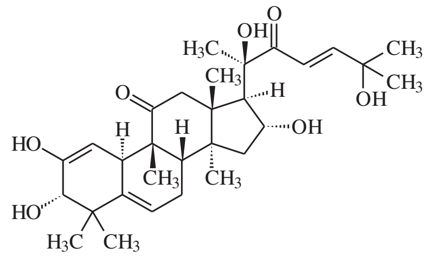
8-232, cucurbitacin B, R = $COCH_3$
cucurbitacin D, R = H



8-233, cucurbitacin C



8-234, cucurbitacin E, R = $COCH_3$
cucurbitacin I, R = H



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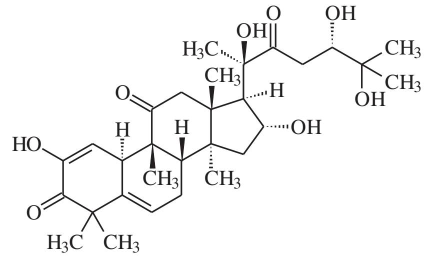
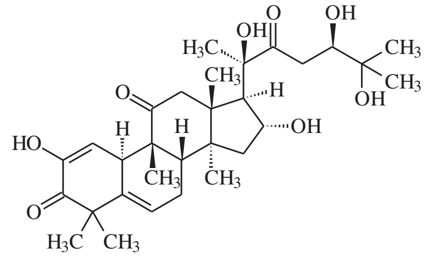
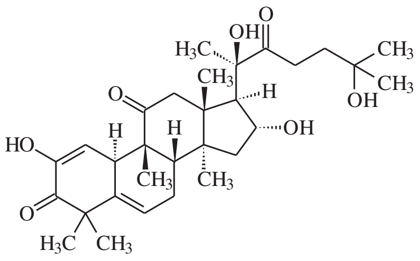


8-236, cucurbitacin J



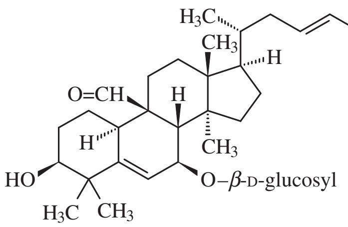
8-237, cucurbitacin K



8-238, cucurbitacin L

about 0.1 mg/l. The highest concentrations of cucurbitacin C in the bitter fruit of cucumbers are found in parts adjacent to the stem (up to 10 mg/kg) and just under the skin. Modern hybrid varieties of cucumbers do not synthesise cucurbitacins.

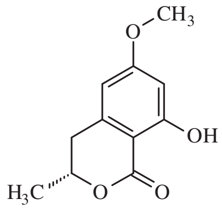
A special group of cucurbitacins are found in bitter melon, also known as bitter gourd or bitter squash (*Momordica charantia*, Cucurbitaceae), which belongs to the most bitter of the edible fruits. The bitter taste of bitter melon is caused by a number of cucurbitane-type triterpenic glycosides called momordicosides, such as momordicosides K and L (**8-239**). Bitter melon originated in India and is now widely cultivated in Asia and Africa. The plant also contains other biologically active compounds for which it is used to treat a number of diseases, such as toothache, diarrhea, furuncle and diabetes.



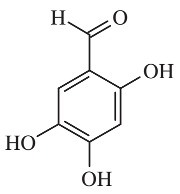
8-239, momordicoside K, R = CH_3
momordicoside L, R = OH

The main contributors to the bitter off-taste of carrots are bisacetylenic oxylipins (see Section 10.3.2.6.5). Infection of carrot roots (*Daucus carota*, Daucaceae) by fungi *Ceratocystis fimbriata*, *Chetomium globosum*, *Botrytis cinerea* and *Thielaviopsis basicola*, produces the bitter phytoalexin (*R*)-6-methoxymellein (8-hydroxy-6-methoxy-3-methyl-3,4-dihydroisocoumarin, **8-240**), which likewise has oestrogenic activity. In carrots marketed in Europe, the levels of 6-methoxymellein ranges from 0.02 to 76.00 mg/kg in fresh carrots and from 0.04 to 15.64 mg/kg in processed carrot products. Levels of 6-methoxymellein are reduced by 69 and 33% in blanched carrots processed by boiling in water or steam treatment, respectively. The recognition threshold concentration of 6-methoxymellein is 20 mg/l. The bitter component of carrot seeds is 2,4,5-trihydroxybenzaldehyde (**8-241**). The bitter taste of some vegetables of the genus *Brassica* (Brassicaceae), such as kohlrabi (*B. oleracea* var. *gongylodes*) and Brussels sprouts (*B. o.* var. *gemmifera*), which sometimes occurs in cooked vegetables, is caused by the presence of goitrin formed by non-enzymatic

decomposition of glucosinolate progoitrin. The bitter taste often occurs during storage of sliced onion (*Allium cepa*). Carriers are probably decomposition products of the lachrymatory factor (*Z*)-thiopropanal sulfoxide that arises from *S*-(prop-1-en-1-yl)cysteine sulfoxide (isoalliin).



8-240, 6-methoxymellein



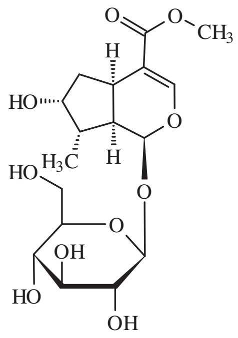
8-241, 2,4,5-trihydroxybenzaldehyde

8.3.5.1.3 Spices and other plant materials

A large number of bitter plants or plant parts (such as roots, rhizomes, bark, leaves, flowers and others) are used as drugs in folk and official medicine, for the manufacture of infusions, tinctures, bitter non-alcoholic and alcoholic beverages, or as a spice for seasoning food.

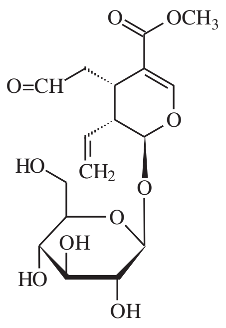
An important bitter substance used for making bitter drinks (such as tonic waters) is the alkaloid quinine, which is obtained from the bark of different species of the genus *Cinchona* (Rubiaceae), especially from the bark of *Cinchona officinalis* native to Amazon rainforest (see Section 10.3.3.1.5).

Many plants used for their bitter taste contain strongly bitter glycosides of **iridoids** (see Section 9.10), which are synthesised by plants as protection against invading microorganisms and grazing herbivores. Iridoids show a number of biological activities (such as cardiovascular, antihepatotoxic, hypoglycaemic, antiviral, anti-carcinogenic and immunomodulatory). For example, the root of *Menyanthes trifoliata* of the Manyanthaceae family contains the bitter iridoid glycoside loganin (**8-242**).



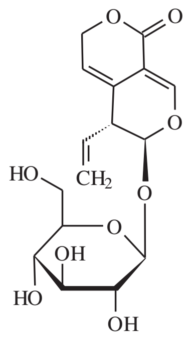
8-242, loganin

Secoiridoids arise from iridoids by cleavage of the cyclopentane ring and other transformations of fission products. Secoiridoid glycosides derived from glycoside of secologanin (**8-243**) occur in the root of yellow gentian (*Gentiana lutea*) and another bitter herb centaury (*Centaurium erythraea* and other plants of the same genus) and almost all plants of the Gentianaceae family.

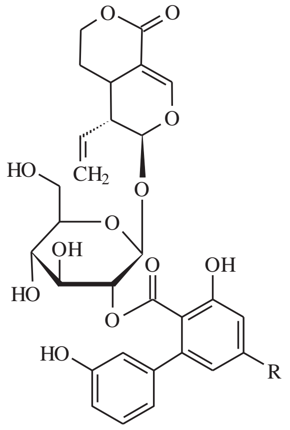


8-243, secologanin

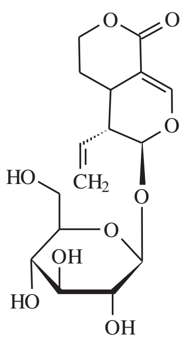
The root of yellow gentian contains several bitter compounds, primarily gentiopicrin (gentiopicroside, **8-244**), amarogentin (amarogentoside, **8-245**) and sweroside (**8-246**). Also present are additional related compounds, such as swertiamarin (swertiamaroside, **8-247**) and deoxyamarogentin (deoxyamarogentoside, **8-245**). Amarogentin is considered the most bitter substance occurring in nature, as its bitter taste is noticeable even at a dilution of 1 : 58 000 000 (which corresponds to a solution prepared by dissolving 17 μg of amarogentin in 1 l of water). The gentiopicrin content in fresh root is 1.5–3.5%, the content of amarogentin is only 0.05%, but due to its low threshold values amarogentin is a significant bitter substance. Swertiamarin is transformed into erythrocentaurin (5-formyl-3,4-dihydroisocoumarin, **8-248**)



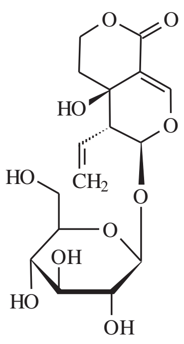
8-244, gentiopicrin



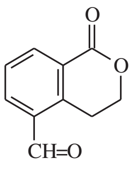
8-245, amarogentin, R = OH
deoxyamarogentin, R = H



9-246, sweroside



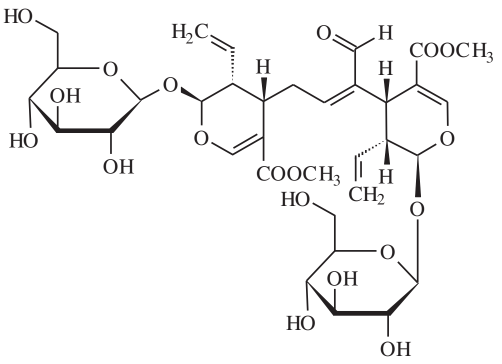
9-247, swertiamarin



8-248, erythrocentaurin

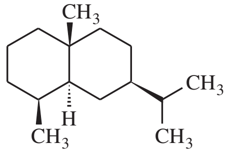
almost quantitatively during isolation of bitter substances in acidic media.

Bitter substances of centaury are similar to bitter substances of yellow gentian. The main component is usually gentiopicin (originally known under the name erytaurin), also present are swertiamarin and amarogentin and other minor bitter substances, such as dimeric centauroside (**8-249**). Some centaury species contain sweroside in almost the same amount as gentiopicrin.

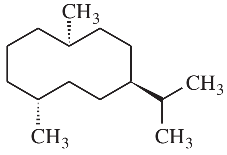


8-249, centauroside

Plants of the family Asteraceae, which are used as a spice and for the production of bitter liqueurs and vermouths (aromatised fortified wine flavoured with various botanicals), contain sesquiterpenic lactones derived from guaiane (**8-226**), eudesmane (**8-250**) and germacrane (**8-251**). Eudesmane (also known as selinene) has the systematic name (*1S*,4a*R*,7*R*,8a*S*)-7-isopropyl-1,4a-dimethyldecahydronaphthalene, germacrane is (1*R*,4*S*,7*S*)-4-isopropyl-1,7-dimethylcyclodecane.

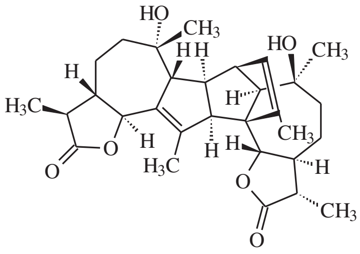


8-250, eudesmane

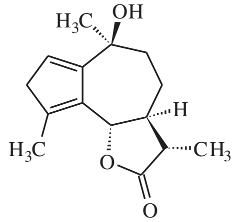


8-251, germacrane

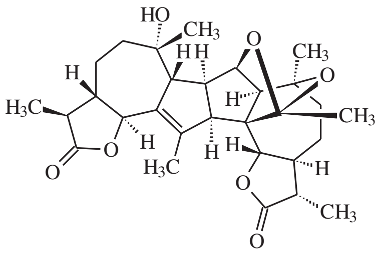
The main bitter component of wormwood (*Artemisia absinthium*) is a dimeric sesquiterpenic lactone absinthin (**8-252**), which is produced by intramolecular cycloaddition (Diels–Alder reaction) probably of two molecules of astabisin (**8-253**), which is derived from guaiane. In wormwood extracts, absinthin is transformed into isomeric anabsinthin (**8-254**). The bitter taste of mugwort (*A. vulgaris*) is derived primarily from the related compounds estafiatin (**8-255**) and balchanin (**8-256**), which are derived from eudesmane. Another bitter substance, which occurs in different plants of the genus *Artemisia*, for example, *A. absinthium*, *A. vulgaris*, *A. pontica* (Roman wormwood) and *A. maritima* (see wormwood) is santonin (**8-257**), which is also derived from eudesmane. It has toxic effects, and therefore safe maximum levels have



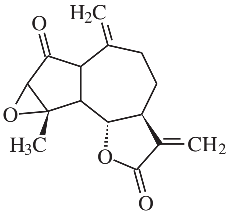
8-252, absinthin



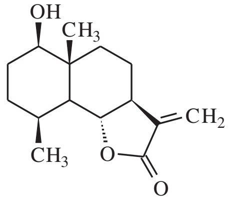
8-253, astabisin



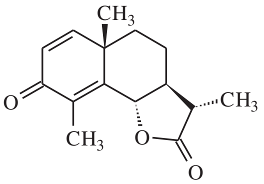
8-254, anabsinthin



8-255, estafiatin



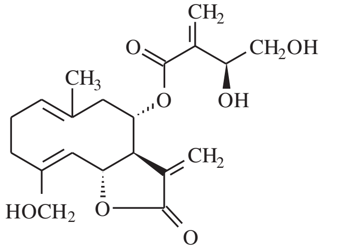
8-256, balchanin



8-257, santonin

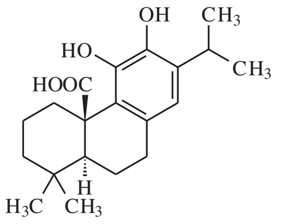
been set for its presence in alcoholic beverages and foods. Toxic components of wormwood essential oil and of some other essential oils are α- and β-thujones (see Section 8.2.13.2).

Another member of the family Asteraceae is St. Benedict's thistle, also known as the blessed or holy thistle (*Cnicus benedictus*), which contains bitter cnicin (**8-258**) derived from germacrane.

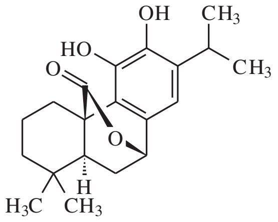


8-258, cnicin

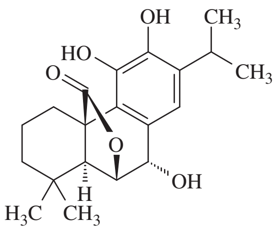
Common sage and rosemary (see Table 8.32), plants of the Lamiaceae family, contain the diterpenes carnosic acid, also known as rosmaricin (**8-259**), derived from *ent*-caurene, and bitter carnosol (picrosalvin, **8-260**), which are potent antioxidants. Carnosic acid is a major component of fresh rosemary tops (1–2%), but is unstable and is enzymatically transformed into carnosol. These two diterpenoids represent about 15% w/w of plants haulm extracts and exhibit about 90% of extract antioxidant activity. Other transformation products of carnosic acid are rosmanol (7α-hydroxy derivative, **8-261**), epirosmanol (7β-isomer, **8-262**) and similar compounds.



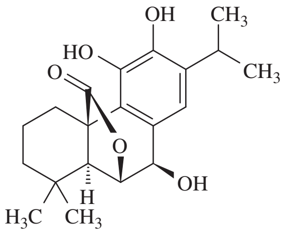
8-259, carnosic acid



8-260, carnosol



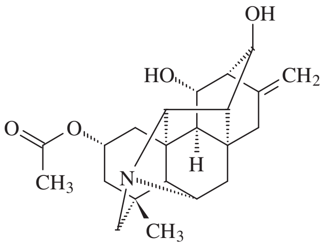
8-261, rosmanol



8-262, epirosmanol

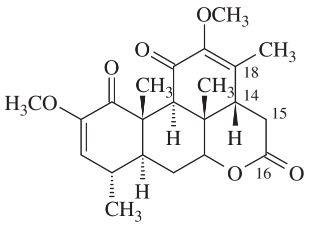
Sweet flag (calamus) rhizome (*Acorus calamus*, Araceae) essential oil contains as the main component toxic β-asaron (see Section 8.2.3.1.2). Other important calamus constituents are bitter alkaloids, of which the most important compound is a glycoside called acorin (**8-263**).

Typical bitter substances of shrubs of the family (Simaroubaceae) are degraded triterpenoids **decanortriterpenoids** related to



8-263, acorin aglycone

limonoids that are called **quassinoids**. The so-called bitter quassia wood from shrubs *Quassia amara*, native to tropical America, contains as the main bitter substance quassin (**8-264**), which is one of the bitterest natural substances. For toxicological reasons (negative effect on fertility of tested animals), flavourings and food ingredients with flavouring properties produced from the source material (and also from the West Indian tree *Picrasma excelsa* of the same family) may only be used for the production of beverages and baked products. Examples of other bitter substances of *Quassia amara* are neoquassin (with a reduced oxo group in position C-16), 14,15-dehydroquassin and 18-hydroxyquassin (with a methyl group in position C-18 replaced by a hydroxymethyl group).



8-264, quassin

The most precious and expensive spice in the world is saffron (dried stigmas of the saffron flower, *Crocus sativus*, Iridaceae), which is used for its bright orange–yellow colour and intense flavour. The bitter substance of saffron picrocrocin is the β-D-glucoside of (*R*)-4-hydroxy-2,6,6-trimethylcyclohex-1-ene-1-carbaldehyde, known as (*R*)-4-hydroxy-β-cyclocitral. Picrocrocin is produced by oxidative cleavage of the carotenoid pigment zeaxanthin by dioxygenase and glycosylation of (*R*)-4-hydroxy-β-cyclocitral (Figure 8.91). Fragments of carotenoids, such as (*R*)-4-hydroxy-β-cyclocitral are called apocarotenoids.

8.3.5.1.4 Other foods and drinks

Dairy products and protein hydrolysates

Precursors of bitter substances in milk and dairy product foods are quite often proteins and mineral salts. Bitter substances are some peptides produced by enzyme proteolysis (see Section 2.3.3.2.1). Bitterness is especially typical for certain dairy products such as cheeses, yoghurt and casein hydrolysates (see Section 2.3.3.2). For example, the bitterness of ripened Gouda cheese was found to be primarily induced by calcium ($CaCl_2$) and magnesium ($MgCl_2$)

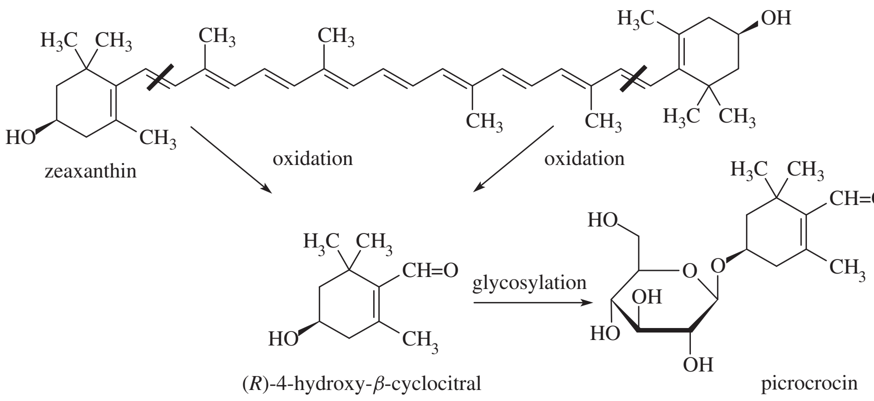


Figure 8.91 Formation of picrocrocin from zeaxanthin.

chlorides, as well as various bitter-tasting free amino acids, whereas bitter peptides were found to influence the bitterness quality rather than the bitter intensity of the cheese. Bitter substances are also formed during pyrolysis of proteins and in reactions of proteins with sugars (in the Maillard reaction).

Legumes and oilseeds

Carriers of bitter and astringent taste of the seeds of plants of the family Fabaceae, for example of certain varieties of pea (*Pisum sativum*) and peanuts (*Arachis hypogaea*) are saponins (see Section 10.3.2.2). The main saponin is sojasaponin B_b (also known as sojasaponin I). The content of saponins in sweet varieties typically ranges between 0.1 and 0.2%, but a higher content is perceived as bitter taste. The content of saponins in peanuts may range from 0.01 to 1.6%. The characteristic bitter and astringent tastes of soy flour (*Glycine max*) are mainly due to isoflavones and their glycosides (daidzein, genistin and glycitein-7-*O*-β-D-glucoside). Compounds responsible for the bitter taste may also be saponins, whose content can range from 0.2 to 5.6%.

The bitter taste of the extraction meal and flour from rapeseeds (*Brassica napus*, Brassicaceae) is caused by sinapine (present as sinapine chloride) and its components choline chloride and sinapic acid, which show about 80% of the sinapine bitterness. Goitrin may also act as a bitter substance as in some *Brassica* vegetables (e.g. in Brussels sprouts).

Hops and beer

Hops (*Humulus lupulus*, Cannabinaceae) are an ingredient with a critical impact on the bitter taste and characteristic odour of beer. Hops also form insoluble complexes with proteins and polypeptides, which contribute to the colloidal stability of beer, the stability of beer foam, and assure the bacteriological stability of beer. Hops are added to the wort, which is boiled for at least 1 hour. After cooling and removal of spent hops, yeast is added to the hopped wort to convert sugars into ethanol and carbon dioxide.

Hops accumulate bitter substances called **bitter acids** in the mature female inflorescences (cones). The level of bitter substances usually amounts to about 5–20% of the cones weight (water content is about 11%). The bitter acids in fresh hops are **phloroglucinols**, derivatives of 1,3,5-benzenetriol, containing a residue of carboxylic acid (acyl) and two to three 3-methylbut-2-en-1-yl (prenyl) side chains as substituents of the aromatic ring. They are therefore prenylated acylphloroglucinol derivatives and, according to the biochemical origin, they are ketides. The so-called hop resins (non-bitter and bitter compounds extractable from hops with diethyl ether and soluble in methanol) were originally divided into:

- soft resins (soluble in hexane), which included α-acids and β-acids;
- non-specific soft resins or resupones that are further divided into α-resupones and β-resupones;
- γ-hard resins (compounds insoluble in hexane) that included water-soluble δ-resins and water-insoluble ε-resins.

At present, the hop bitter acids (soft resins) are divided into two groups:

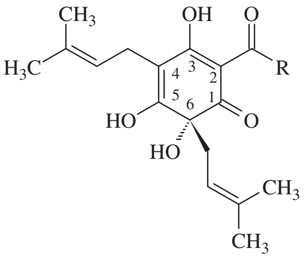
- humulone homologues called humulones, α-acids or α-bitter acids (with two prenyl substituents);
- lupulone homologues called lupulones, β-acids or β-bitter acids (with three prenyl substituents).

The basic humulone homologues (**8-265**) are cohumulone and adhumulone and minority components are prehumulone and posthumulone. Analogously, the main lupulone homologues (**8-266**) are colupulone, adlupulone, prelupulone and postlupulone. These acids are bitter only by name, but in fact they have an indifferent taste and are almost insoluble in water and have pronounced bacteriostatic activity. The approximate composition of these two groups of bitter acids in different hop varieties is shown in Table 8.43. Their content is about 25% of the dry weight of the hop cones.

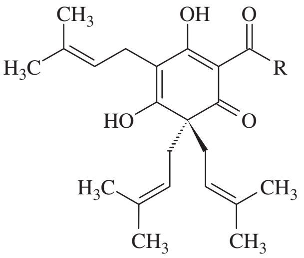
Both types of hop bitter acids are very reactive compounds, in particular in the light. During the drying, storage and brewing of hops they isomerise (also in aqueous solutions), oxidise and

Table 8.43 α- and β-bitter acids contents in hops.

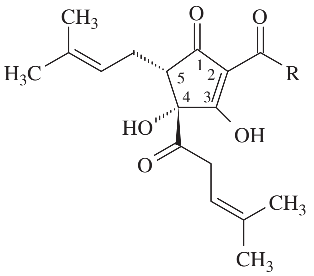
Compound	Content (% of α-bitter acids)	Compound	Content (% of β-bitter acids)
α-Bitter acids		***β-Bitter acids***	
Humulone	35–70	Lupulone	30–55
Cohumulone	20–65	Colupulone	37–68
Adhumulone	10–15	Adlupulone	11–12
Prehumulone	1–10	Prelupulone	1–5



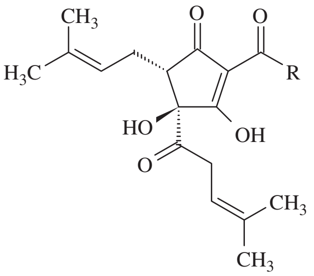
8-265, α-bitter acids
humulone, R = $CH_2CH(CH_3)_2$
cohumulone, R = $CH(CH_3)_2$
adhumulon, R = $CH(CH_3)CH_2CH_3$
prehumulon, R = $CH_2CH_2CH(CH_3)_2$
posthumulon, R = CH_2CH_3



8-266, β-bitter acids
lupulone, R = $CH_2CH(CH_3)_2$
colupulone, R = $CH(CH_3)_2$
adlupulone, R = $CH(CH_3)CH_2CH_3$
prelupulone, R = $CH_2CH_2CH(CH_3)_2$
postlupulone, R = CH_2CH_3



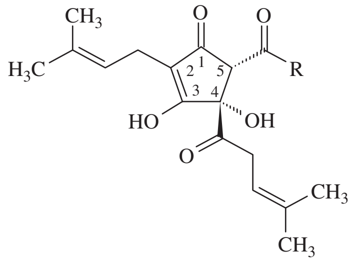
8-267, *cis*-isohumulones



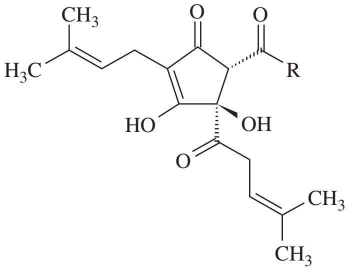
8-268, *trans*-isohumulones

polymerise into a large number of different products. Boiling the wort transforms a part of humulones into isohumulones known as iso-α-acids or iso-α-bitter acids. Their taste perception threshold is about 7 mg/l in water. The remaining α-acids seem not to have any major impact on the taste of the final product. The isomerisation reaction proceeds as a stereoselective tautomerisation of enolate into ketone followed by ring contraction (at C-5 and C-6) of acyloins. Iso-α-acids have two chiral centres and therefore exist as (4*R*,*Z*)-isomers (*cis*-isomers) (**8-267**) and (4*S*,*E*)-isomers (*trans*-isomers) (**8-268**) depending on the spatial arrangement of the tertiary alcohol function at C-4 and the prenyl side chain at C-5. The ratio of these two isomers in wort is about 2 : 1 in favour of the *cis*-isomers. Six major iso-α-acids, namely *cis*-isohumulone, *cis*-isoadhumulone, *cis*-isocohumulone, *trans*-isohumulone, *trans*-adhumulone and *trans*-isocohumulone generally occur in beers at concentrations of 15–80 mg/l or even 100 mg/l in very bitter English ales. Oxoforms of humulones contain a double acyloin system, so by the contraction at C-1 and C-6 of acyloin they produce, to a lesser extent, the so-called anti-isohumulones (anti-iso-α-acids or anti-iso-α-bitter acids), their *cis*-isomers (**8-269**) and *trans*-isomers (**8-270**) as isomerisation products, respectively.

Iso-α-acids are moderately strong acids with pK_a values around 3 (beers of Pilsner type have pH around 5.0–5.2), more soluble than the corresponding humulones (pK_a values are around 5.5) and have an intensely bitter taste. According to the older classification, iso-α-acids are α-resupones, the most important bitter constituents of beer, stabilising the foam and exhibiting bacteriostatic effects.



8-269, *cis*-anti-isohumulones

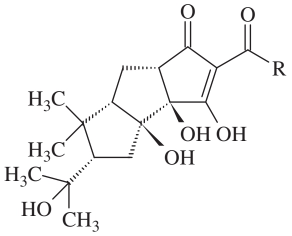


8-270, *trans*-anti-isohumulones

In direct sunlight or in the presence of photosensitisers (such as riboflavin and polyphenols), the side chain is cleaved, which gives dehydrohumulinic acids (Figure 8.92). Decarbonylation of the formed acyl radical and recombination with a thiol radical, originating from sulfur compounds in beer, yields but-2-en-1-thiol, causing the so-called light-struck off-flavour (Section 8.2.9.1.2).

It has been demonstrated that the aging of beer in brown bottles induces a preferential acid-catalysed degradation of the *trans*-isomers of iso-α-acids, whereas the corresponding *cis*-isomers are more stable. The tricyclic reaction products generated from *trans*-iso-α-acids include the so-called tricyclohumol, tricyclocohumol and tricycloadhumol (**8-271**). In stored hops similar oxidation products of humulones with a cyclised side chain, such as tricyclodehydroisohumulones (**8-272**) have been found. Their bitterness is about 70% of the bitterness of isohumulones. Other products are dihydropyrans (**8-273**) and 2-acyltetronic acids (**8-274**). The storage of beer in polyethylene terephthalate bottles, permeable to oxygen, induces autoxidation of both isomers to *cis*- and *trans*-hydroxyalloiso-α-acids via the corresponding free radicals and *cis*- and *trans*-hydroperoxides (Figure 8.93).

On alkaline treatment, isohumulones are transformed into (4*R*,*Z*)- and (4*S*,*E*)-humulinic acids, also known as *cis*-humulinic



8-271, tricyclohumol, R = $CH_2CH(CH_3)_2$
tricyclocohumol, R = $CH(CH_3)_2$
tricycloadhumol, R = $CH(CH_3)CH_2CH_3$

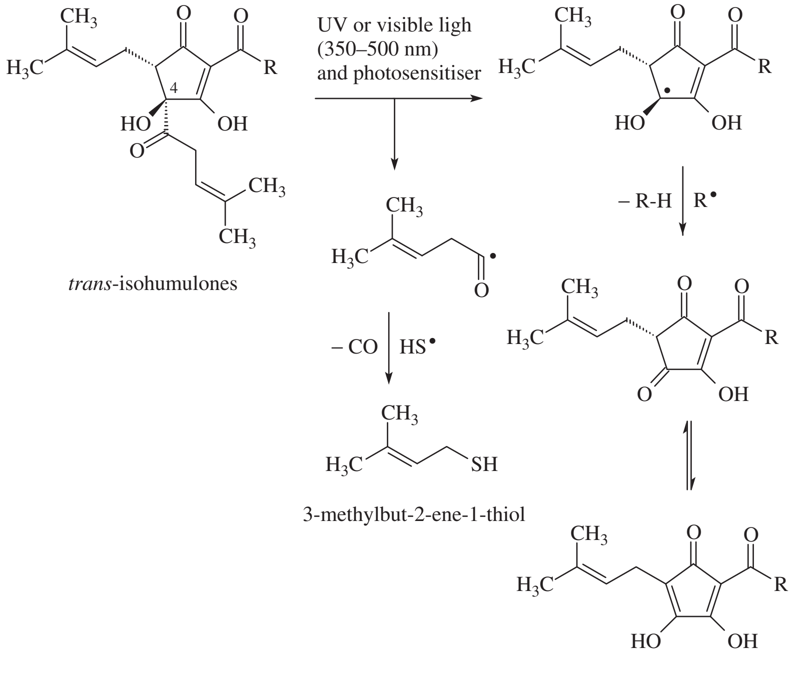
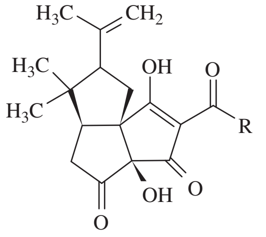
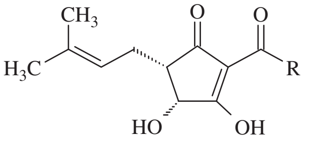


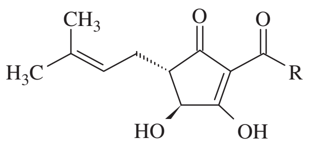
Figure 8.92 Formation of dehydrohumulinic acids and 3-methylbut-2-ene-1-thiol from isohumulones.



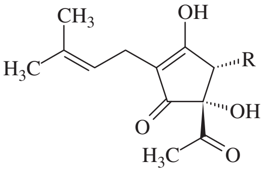
8-272, tricyclodehydroisohumulone



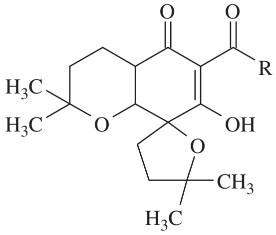
8-275, *cis*-humulinic acids



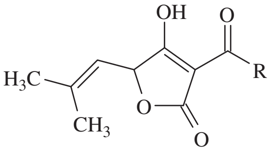
8-276, *trans*-humulinic acids



8-277, *cis*-anti-acetylhumulinic acids



8-273, hops dihydropyrans



8-274, hops 2-acyltetronic acids

acids (**8-275**) and *trans*-humulinic acids (**8-276**), respectively, and to other products. Related anti-acetylhumulinic acids (**8-277** and **8-278**) are considered the bitterest substances in hops.

β-Bitter acids do not isomerise during the wort boiling and contribute more to the aroma than bittering. Oxidative decomposition

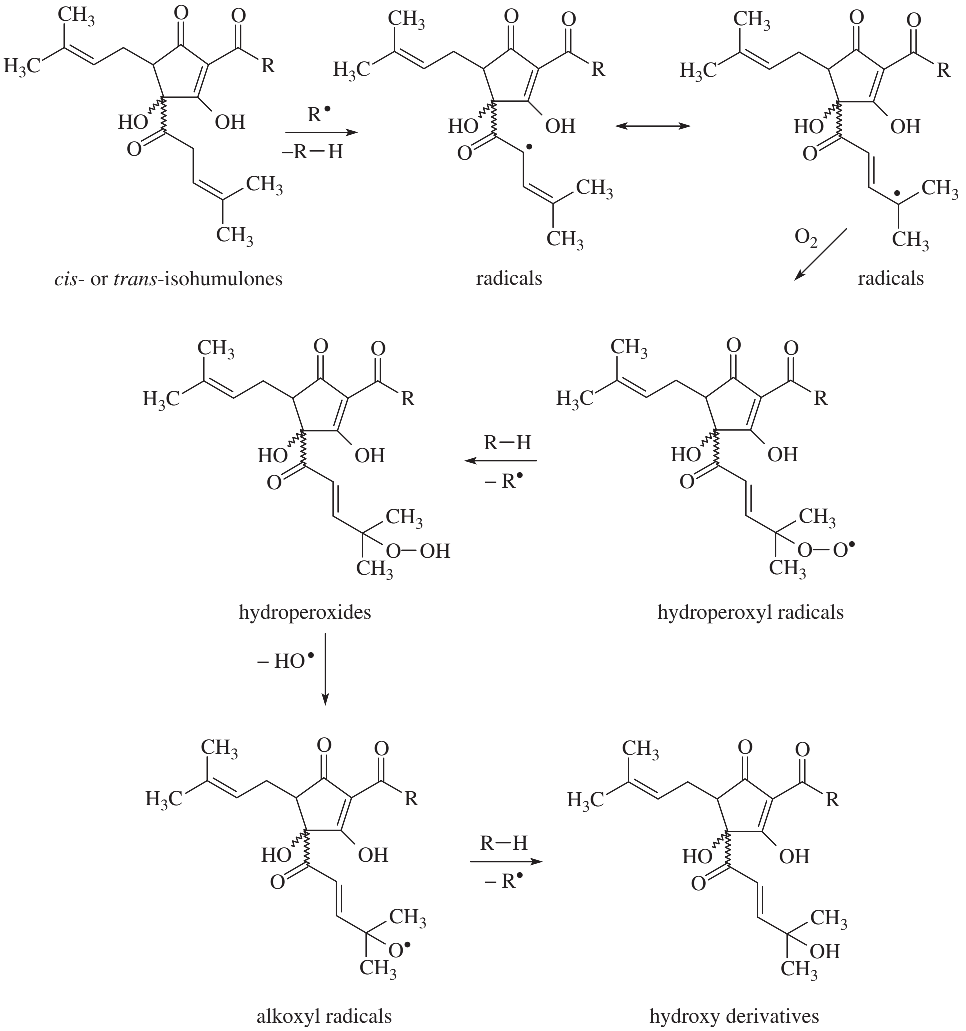
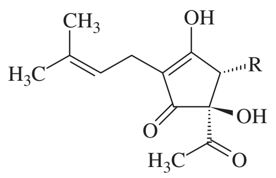
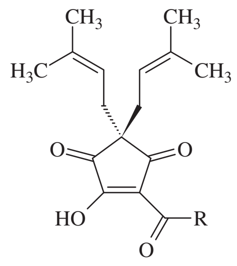


Figure 8.93 Oxidation of isohumulones. Intelmann and Hofmann, 2010, fig 7. Reproduced by permission of the American Chemical Society.

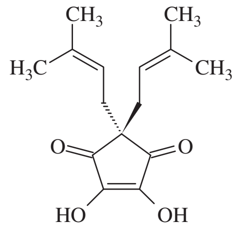


8-278, *trans*-anti-acetylhumulinic acids

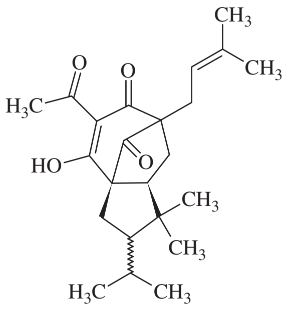
of β-bitter acids is easier and most oxidation reaction products possess unpleasant organoleptic characteristics. Therefore, β-bitter acids are considered a negative factor in brewing and hop varieties with low content of lupulones are preferred. The main bitter transformation products of lupulones are hulupones (such as hulupone, cohulupone and adhulupone, **8-279**), which belong to traditionally recognised β-resupones, hulupinic acids (**8-280**), the corresponding tautomers luputriones, tricyclolupones (**8-281**), nortricyclolupones (**8-282**) and dehydrotricyclolupones (**8-283**).



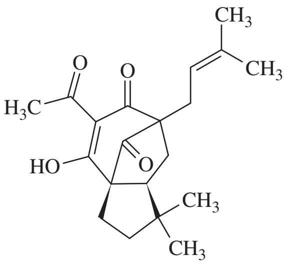
8-279, hulupones



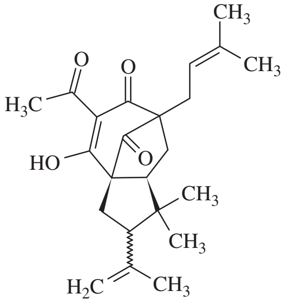
8-280, hulupinic acids



8-281, tricyclolupones

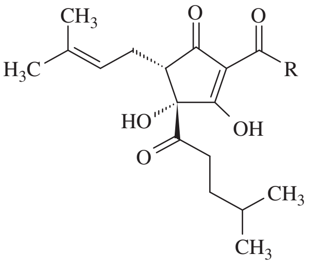


8-282, nortricyclolupones

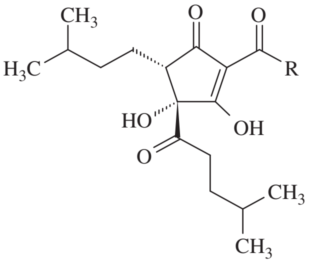


8-283, dehydrotricyclolupones

Easy oxidation of humulones and lupulones in hops is the reason for the use of various hop products, among others, hydrogenated (reduced) iso-α-acids. Examples of hydrogenated substances are *cis*-dihydroisohumulone (**8-284**) and *cis*-tetrahydroisohumulone (**8-285**), the taste threshold of which is 0.007 mg/l in water. Dihydro- and tetrahydro-iso-α-acids are stable even in the light and do not undergo photofragmentation. Beers containing these compounds can be stored in white glass bottles. Dihydroisohumulones are also known as rho-isohumulones. The bitter taste of roasted malt used in the production of dark beers is caused by the presence of 2,5-dioxopiperazines and other products of the Maillard reaction. In comparison with the bitterness of bitter substances of hops, the contribution of these compounds to the bitterness of beer is rather questionable.



8-284, *cis*-dihydroisohumulones



8-285, *cis*-tetrahydroisohumulones

In addition to bitter substances, hop cones contain a number of monoterpenes and sesquiterpenes (0.5–3%) that carry their own characteristic aroma. Some non-volatile components present in the hop, such as polyphenols (3–6%), contribute to a full mouth feel during beer tasting. Important non-volatile components of hop cones are also prenylated flavonoids that show oestrogenic and anti-carcinogenic effects.

Ciders and wines

The bitter and astringent taste of cider from specific varieties of bitter apples is caused by the presence of oligomeric procyanidins, mainly tetramers and higher oligomers. Procyanidins sometimes cause the bitter taste of grape wines, especially white wines.

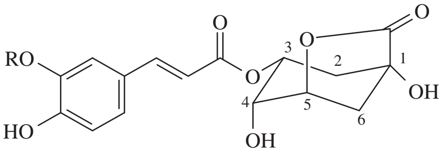
Tea

The bitterness and astringency of tea is related primarily to the presence of phenolic compounds, especially the so-called catechins,

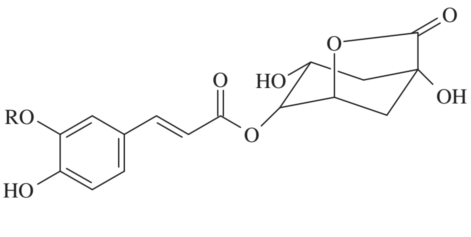
which constitute 10–30% of dry green tea leaves. Black tea contains pigments, mainly theaflavins and thearubigins, which are formed from catechins during fermentation of the tea leaves. Caffeine also plays an important role, particularly in its interaction with phenolic compounds. Additionally involved in the bitter taste of tea are some amino acids (especially L-theanine) and saponins with characteristic aglycones (sapogenols), such as theasapogenols, assamsapogenols and related compounds (see Section 10.3.2.2).

Coffee

The bitterness of roasted coffee is affected by roasting the coffee beans (the degree of bitterness increases with prolonged roasting), preparation of brine, water hardness, temperature and use of sugar and milk. The bitterness of coffee is due to many compounds. The majority of the bitter taste is shown by the transformation products of chlorogenic acids formed during the roasting of the coffee seeds by dehydration, decarboxylation and subsequent reactions. Chlorogenic acids partially dehydrate to form lactones. For steric reasons, the main lactones of roasted coffee are γ-lactones derived from 3-*O*-cinnamoyl-L-quinic (chlorogenic) and 4-*O*-cinnamoyl-L-quinic (cryptochlorogenic) acids. The main products are 3-*O*-L-caffeoyl-quino-1,5-lactone (also known as 3-*O*-caffeoyl-γ-quinide, **8-286**), which are produced from chlorogenic acid, and 4-*O*-caffeoyl-L-quino-1,5-lactone (4-*O*-caffeoyl-γ-quinide, **8-287**) formed from cryptochlorogenic acid. These γ-lactones are followed by γ-lactones derived from 3-*O*- and 4-*O*-feruloyl-L-quinic acid and from other depsides.

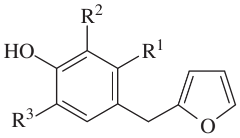


8-286, 3-*O*-caffeoyl-L-quino-1,5-lactone, R = H
3-*O*-feruloyl-L-quino-1,5-lactone, R = CH_3



8-287, 4-*O*-caffeoyl-L-quino-1,5-lactone, R = H
4-*O*-feruloyl-L-quino-1,5-lactone, R = CH_3

Reactions of chlorogenic acids during coffee roasting are very complex. Along with dehydration, chlorogenic acids are also decarboxylated and hydrolysed during coffee roasting. For example, 5-*O*-caffeoyl-L-quinic (neochlorogenic) acid yields, by hydrolysis, caffeic acid, which on decarboxylation or *syn*-elimination of quinic acid gives 4-vinylcatechol. Protonation of 4-vinylcatechol gives the reactive electrophilic cation with a quinone methide structure, which is condensed with 4-vinylcatechol and by subsequent reactions produces a group of polyhydroxylated phenylindans, such as 1,3-bis(3′,4′-dihydroxyphenyl)butane, *trans*-1,3-bis(3′,4′-dihydroxyphenyl)but-1-ene, *cis*- and *trans*-5,6-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane and *cis*- and *trans*-4,5-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane, which exhibit a harsh and lingering bitter taste profile. Subsequent reactions with other 4-vinylcatechol cations yield higher oligomers. Only *cis*-isomers of indanes are listed (Figure 8.94). Dihydroxybenzene and trihydroxybenzene derivatives arising by decarboxylation of phenolic acids, such as catechol (pyrocatechol), 3-methyl- and 4-methylcatechol and pyrogallol, condense with furan derivatives resulting from the Maillard reaction from sugars (such as furfuryl alcohol) to form bitter (furan-2-yl)methylated benzene diols and triols. Compounds of this group identified in coffee included 4-(furan-2-ylmethyl)benzene-1,2-diol formed from catechol, 3-(furan-2-ylmethyl)-6-methylbenzene-1,2-diol formed from 3-methylcatechol, 4-(furan-2-ylmethyl)-5-methylbenzene-1,2-diol formed from 4-methylcatechol and 4-(furan-2-ylmethyl)benzene-1,2,3-triol, which arises from pyrogallol (**8-288**).



8-288, (furan-2-ylmethyl)benzene diols and triols
4-(furan-2-ylmethyl)benzene-1,2-diol, $R^1 = R^2$ = H, R^3 = OH
3-(furan-2-ylmethyl)-6-methylbenzene-1,2-diol, $R^1 = CH_3$, R^2 = OH, R^3 = H
4-(furan-2-ylmethyl)-5-methylbenzene-1,2-diol, $R^1 = CH_3$, R^2 = H, R^3 = OH
4-(furan-2-ylmethyl)benzene-1,2,3-triol, $R^1 = R^2$ = OH, R^3 = H

Quinic acid resulting from chlorogenic acids also has a bitter taste, but the contribution of 2,5-dioxopiperazines (see Section 2.3.3.2.1) and other heterocyclic compounds generated during roasting by the Maillard reaction is not very significant.

Cocoa

The bitterness of cocoa, like the bitterness of coffee, increases with the degree of roasting. The main bitter components are bitter purine alkaloids theobromine and caffeine, as well as cyclic dipeptides (2,5-dioxopiperazines) formed by thermal fragmentation of proteins and products of the Maillard reaction.

8.3.5.2 Methods of debittering and masking the bitter taste

In certain foods a bitter taste is definitely not desirable, therefore different debittering methods have been developed. Methods for removing the bitter taste of enzymatic protein hydrolysates (such as casein hydrolysates) are described in Section 2.3.2.2. These methods are mainly based on controlled proteolysis, plastein reaction, extraction with azeotropic mixtures of alcohols and masking of bitter substances.

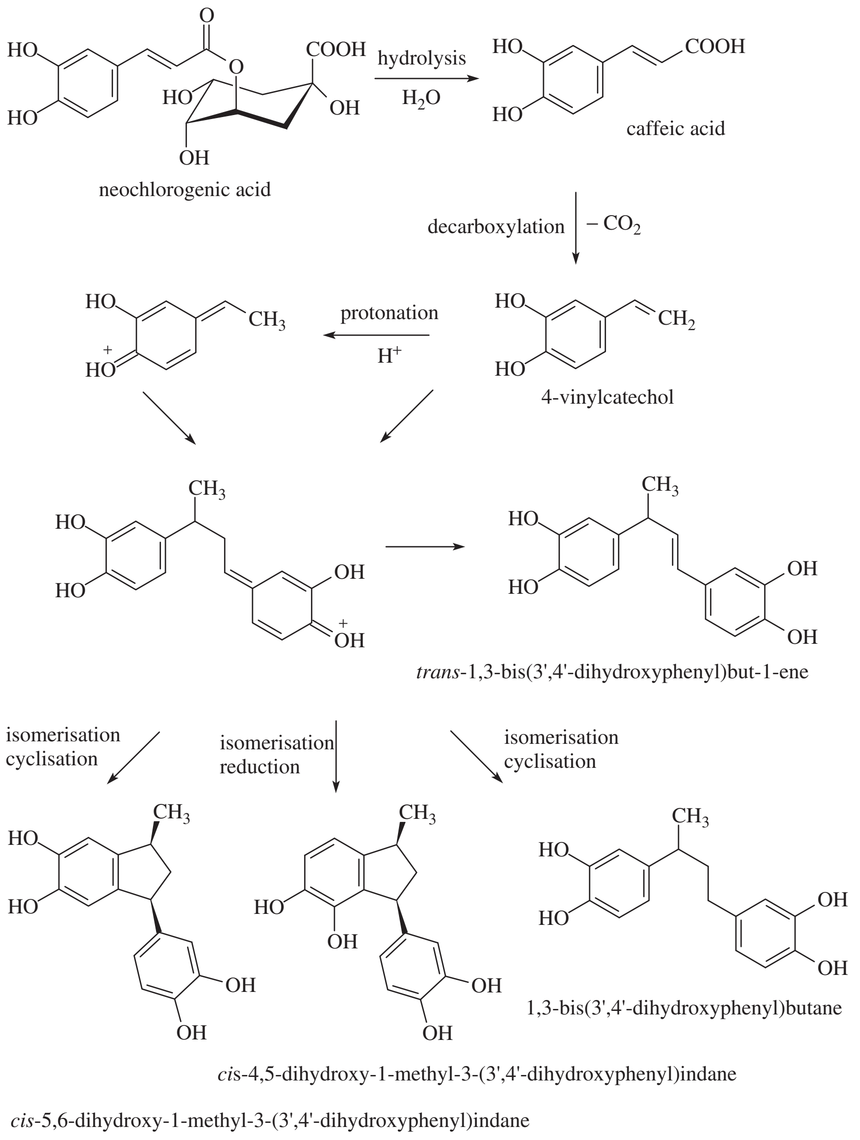


Figure 8.94 Formation of bitter substances from neochlorogenic and caffeic acids.

Debittering of citrus (grapefruit) juices that contain bitter flavanone-7-glucosides is based on enzymatic hydrolysis of the sugar residue in bitter glycosides. The necessary enzymes exhibiting the activities of α-rhamnosidase and β-glucosidase are derived from microorganisms *Phomopsis citri*, *Cochliobolus miyabeanus* and others. Debittering of orange juice (removal of bitter limonin) is done using special enzymes or immobilized cells (such as bacteria *Arthrobacter globiformis*).

Debittering of olives is based on hydrolysis of the bitter oleuropein by fermentation or in alkaline solutions. Hydrolysis of oleuropein in immature green or yellow fruits is done by dipping in a 1.3 to 2.6% sodium hydroxide solution for 6–10 hours. To obtain black unfermented olives from ripe blue olives that are no longer bitter, the fruits are repeatedly soaked in 1–2% NaOH solution with aeration. Under these conditions intense non-enzymatic browning reactions occur, and the dark colour of the fruits is stabilised by the addition of ferrous gluconate that forms black complexes with oxidised polyphenols.

8.3.5.3 Physiology, nutrition and use

Some bitter substances exhibit different physiological effects and may even be highly toxic (e.g. some alkaloids). The bitter taste of food is so often unconsciously associated with the presence of toxic substances, for example in mushrooms, potatoes and lupine seeds. Physiological effects of the bitter substances such as alkaloids, cyanogenic glycosides, saponins, degradation products of glucosinolates (such as goitrin) and plant phenols are discussed in Chapter 10, along with health assessment and appropriate legislation. A number of other bitter compounds have beneficial physiological effects as they act as antioxidants (e.g. many phenolic compounds), and some as anticarcinogens (such as cucurbitacins

and many phenolics). Bitter substances generally support the appetite, which lies in the increased secretion of gastric juices and improved food digestion.

Bitter substances harmless to health have found use mainly in the manufacture of bitter soft drinks, such as bitter lemon (a carbonated drink flavoured with quinine and lemon) and tonic water (a carbonated water flavoured with quinine) and alcoholic beverages, such as beer, specialty wines (vermouths), liqueurs and appetisers.

8.3.6 Astringent substances

Astringency is often accompanied by bitter or sour, or both, taste properties. The different sub-qualities perceived can be caused by different astringent compounds and influences by other food constituents, such as acids and sugars. The primary cause of astringency is the interaction of salivary proteins with certain polymeric phenolic compounds present in foods of plant origin. These interactions lead to denaturation of saliva proteins, loss of their protective effects and astringent compounds may thus consequently interact with the oral cavity proteins, which are perceived as astringency. In addition to phenolic compounds, other compounds can likewise contribute to the astringent taste of food commodities (see Section 8.3.6.5).

Phenolic compounds interacting with proteins are collectively called **tannins**. The common feature of tannins is their ability to react with proteins and precipitate them from aqueous solutions. This reaction is applied *in vivo* in the regulation of enzymatic functions of plant proteins in damaged tissues (structurally similar lignins are missing this feature). The affinity of tannins for proteins depends on the amount of hydroxyl groups and their arrangement, degree of polymerisation of phenols, primary, secondary and tertiary structure of proteins and other factors. In the formation of complexes with proteins, tannins primarily interact via hydrogen bonds and hydrophobic interactions. Particularly strong hydrogen bonds exist between hydroxyl groups of phenols and the secondary amino groups of bound proline (saliva proteins are proline rich) and amide groups of peptide bonds. Ionised hydroxyl groups (in solutions of $pH > 10$) do not react with proteins. Low molecular weight phenolic compounds, such as phenolic acids and simple flavonoids, although reacting with proteins, do not form cross-links between polypeptide chains, which does not result in precipitation of proteins.

Tannins are divided into two large groups:

- **hydrolysable tannins**
- **condensed tannins**.

Hydrolysable tannins are polymers of gallic acid esters, they are polygalloyl esters. Condensed tannins, formerly also known as flavolans, are polymers of some flavonoid compounds with the structure of 3-hydroxyflavan (flavan-3-ol). However, virtually any number of combinations of condensed tannins and hydrolysable tannins, called complex tannins similarly exist.

Owing to their properties, tannins have been used for millennia to tan animal hides into leather. As natural food components, tannins are of great importance as they often substantially affect the desirable and also undesirable taste characteristics. A desirable property is a reasonable astringency of tea, coffee, cocoa (added milk or cream removes the astringency as a result of interaction of tannins with milk proteins), red wine and beer. Undesirable astringency is shown by unripe fruits, such as bananas and persimmons (*Diospyros kaki*, Ebenaceae) and unripe walnuts. In some drinks, such as fruit juices, beer and wine, hazes or sediments are formed, which proteins and tannins participate in. Beer and brewing technology distinguishes between a chill haze and a permanent haze. The polyphenols combine slowly with proteins to form a chill haze when beer is cooled, but the haze redissolves when it is warmed up. As the polyphenols polymerise into larger units, they become insoluble at room temperature to form an irreversible haze. A common practice is, therefore, to eliminate tannins in beer and wine by the use of additives, such as gelatine, polyvinylpolypyrrolidone or polyamide.

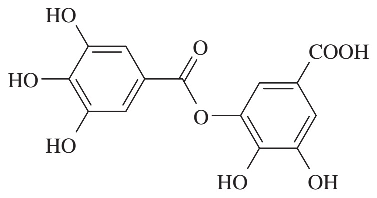
8.3.6.1 Hydrolysable tannins

Hydrolysable tannins are usually divided into:

- **gallotannins**
- **ellagotannins** (or ellagitannins).

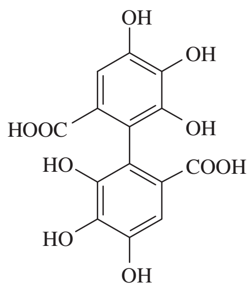
Both groups of hydrolysable tannins are derivatives of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose in which gallic acid and products of its biochemical transformations are bound by ester (depside) bonds to glucose.

Gallic acid can bind through hydroxyl groups other molecules of gallic acid to form chains containing 2–5 gallic acid molecules. The depside formed from two molecules of gallic acid is called *m*-digallic acid (**8-289**). Monosubstituted to pentasubstituted glucoses are often called simple galloyl glucoses, unlike the complex molecules that are called gallotannins. Less common components of gallotannins are some other saccharides, quinic acid and other compounds. Hydrolysable tannins have a relative molecular weight up to about 5 kDa. Hydrolysis of gallotannins by acids, alkalis or esterases (tannase, also known as tannin acyl hydrolase) yields D-glucose and gallic acid.

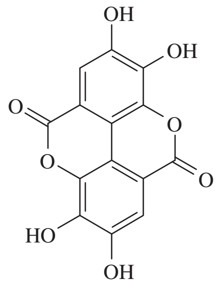


8-289, *m*-digallic acid

Ellagotannins are formed during biosynthesis of hydrolysable tannins by oxidation of two adjacent gallic acid residues and by their combining through covalent bonds. Ellagotannins therefore contain hexahydroxybiphenyl residues derived from the 3,4,5,3′,4′,5′-hexahydroxybiphenylic acid (**8-290**), which arises through their



8-290, 3,4,5,3´,4´,5´-hexahydroxybiphenylic acid

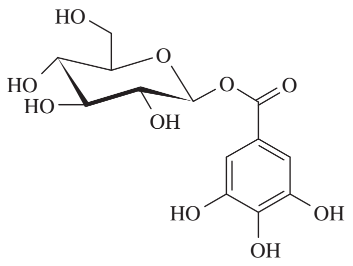


8-291, ellagic acid

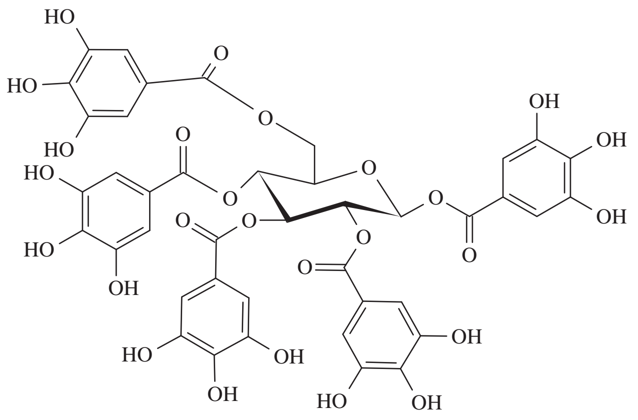
hydrolysis and in acid the hydrolysates spontaneously dehydrate to form ellagic acid (**8-291**).

8.3.6.1.1 Gallotannins

Biosynthesis of gallotannins is based on gallic acid and an activated form of glucose (UDP-glucose). The first intermediate is 1-*O*-galloyl-β-D-glucopyranose (β-glucogallin, **8-292**), which is the donor of the galloyl residue in the biosynthesis of di- to 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranoses (**8-293**). Typical gallotannins are products known commercially as tannin or tannic acid (containing residues of gallic and *m*-digallic acids) that are extracted from oak apples,[6] (*Quercus infectoria*, Fagaceae), tara pods (*Caesalpinia spinosa*, Fabaceae), gallnuts from *Rhus semialata* (Anacardiaceae) or Sicilian sumac leaves (*Rhus coriaria*).



8-292, 1-*O*-galloyl-β-D-glucopyranose



8-293, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucopyranose

Tannic acid is a mixture of esters containing between 3 and 12 molecules of gallic acid, but also *m*-digallic acid and higher depsides. The composition is highly variable depending on the origin of the tannic acid. Tannic acid has found use as a food additive and an agent to prevent the formation of a chill haze in beer and turbidity in wine and vinegar.

8.3.6.1.2 Ellagotannins

Ellagotannins occur as components of commercial tannic acid, various extracts and infusions (e.g. teas from medicinal herbs and bark of trees) and are also natural constituents of some alcoholic beverages matured in oak barrels,[7] for example high quality wines and spirits, such as cognac, brandy, whisky, bourbon and rum. All components extracted from the wood are then degraded to some extent, which gives rise to various phenolic compounds that have a role to play in the flavour-active components of the alcoholic beverage (Table 8.44).

Monomers

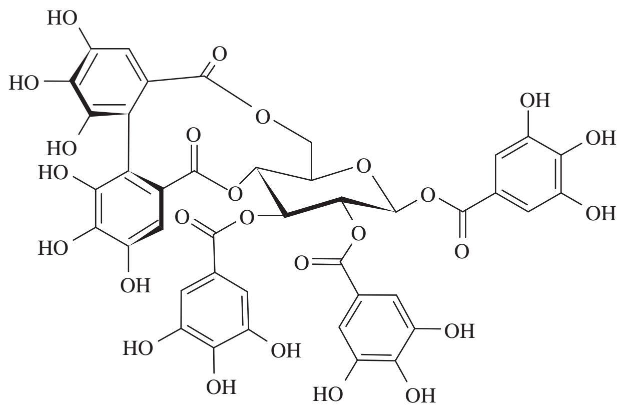
The structures of several hundred simple ellagotannins occurring in nature are known. An example is simple ellagotannin corilagin (**8-294**), which occurs in the leaves of cranberries (*Rhodococcus vitis-idaea*, syn. *Vaccinium vitis-idaea*, Ericaceae). Other examples of ellagotannins found in many plants as monomers or as oligomeric ellagotannins are tellimagrandin I (**8-295**) and tellimagrandin II (**8-296**), which contain one ellagic acid, and pedunculagins (**8-297**) and casuarictin (**8-298**) with two ellagic acids in the molecule. Ellagotannins punicalagins (**8-299**) are found naturally as α- and β-anomers in pomegranate juice at a concentration of about 2 g/l or even higher, depending on the cultivar. Pomegranate ellagotannins

[6]Oak apple is the common name for a large, round (20–50 mm in size) gall commonly found on many species of oak and caused by metabolites injected by the larva of certain kinds of gall wasp, such as *Cynips quercusfolii*, which lay their eggs on oak leaves.

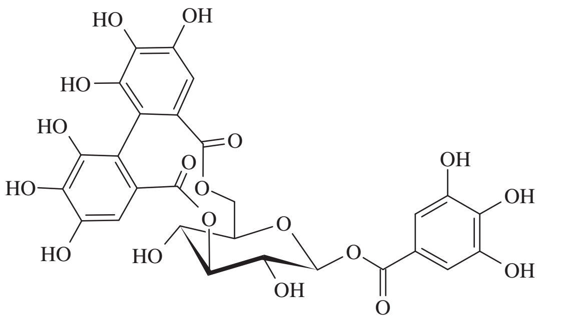
[7]For the production of oak barrels in Europe wood of the English oak (*Quercus robur*) or of the Sessile oak (*Q. petraea*) and in the USA the wood of the white oak (*Q. alba*) are used. In addition to insoluble polymers (cellulose, hemicelluloses and lignin), oak wood contains about 10% of phenolic compounds in dry matter, the predominant part of which are ellagotannins.

Table 8.44 Lignin, ellagotannins and important phenols contents in cognac during aging in oak barrel.

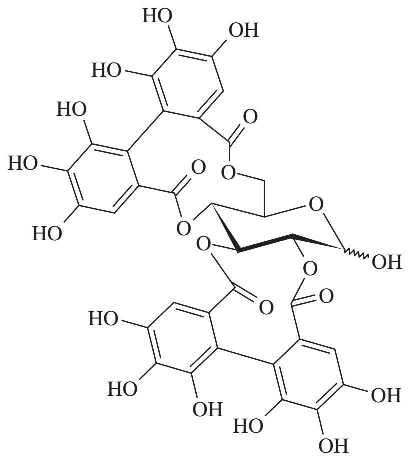
Compound	Content (mg/l)				
	1 year	2 years	10 years	20 years	30 years
Lignin	12	29	127	201	219
Ellagotannins	10	25	31	17	4
Ellagic acid	7	12	32	44	55
Gallic acid	3	3	22	23	26
Vanillin	1	2	6	7	7
Syringaldehyde	1	3	11	13	14
Vanillic acid	1	2	3	4	5
Syringic acid	1	1	4	6	6



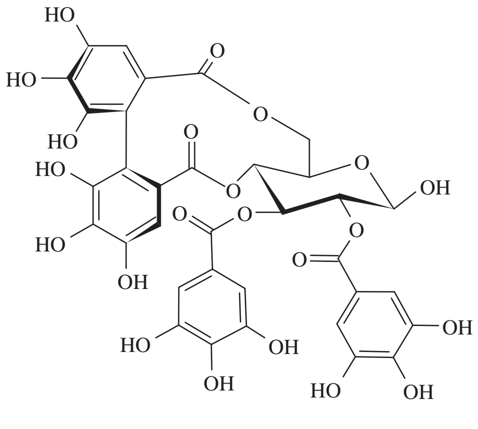
8-296, tellimagrandin II



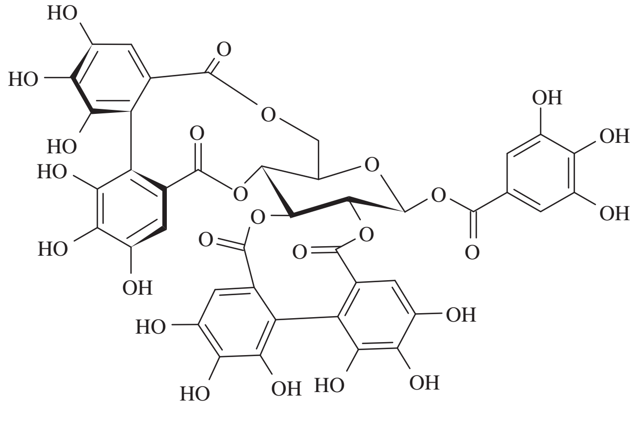
8-294, corilagin



8-297, pedunculagins



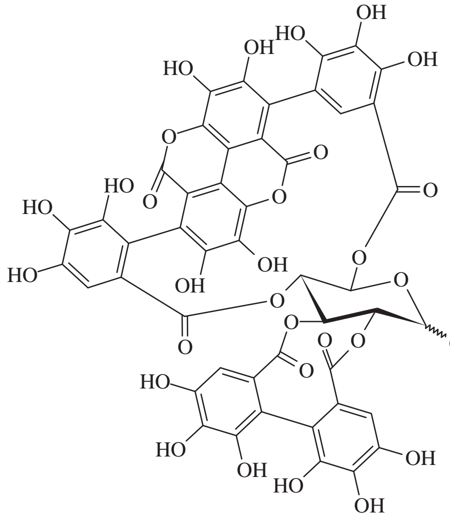
8-295, tellimagrandin I



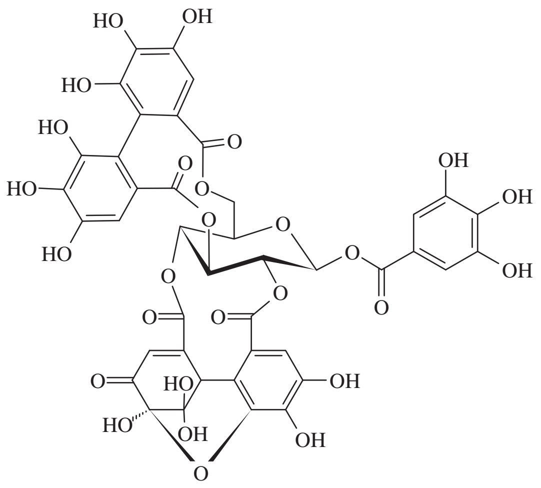
8-298, casuarictin

of similar structure are punicalins with non-esterified C-2 and C-3 glucose hydroxyl groups.

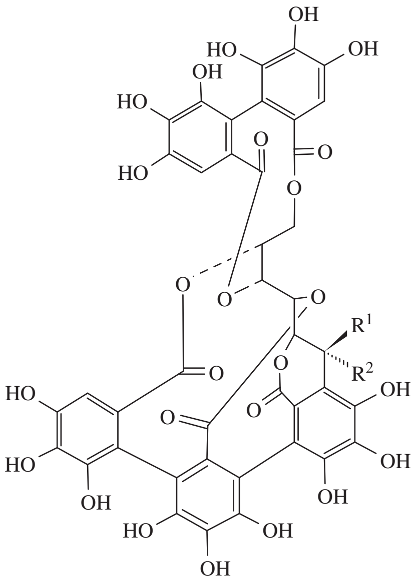
In addition to these compounds, monomers with less common structures also occur in plants, such as geraniin (**8-300**) with dehydrohexahydroxybiphenyl structure, which is classified as dehydroellagotannin. It occurs in many plants of the Geraniaceae and Euphorbiaceae families. Further examples of ellagotannins are the so-called *C*-glucosidic tannins, which are constituents of oak wood. Representatives of these tannins are two diastereoisomers, castalagin and vescalagin (**8-301**). Most of the other known ellagotannins are derived from these two forms.



8-299, punicalagins



8-300, geraniin



8-301, castalagin, R^1 = H, R^2 = OH
vescalagin, R^1 = OH, R^2 = H

Oligomers

Only about 20 monomeric ellagotannins are components of oligomeric ellagotannins that arise from monomers by biochemical reactions (mainly oxidation). The most common oligomers are dimers, trimers and tetramers.

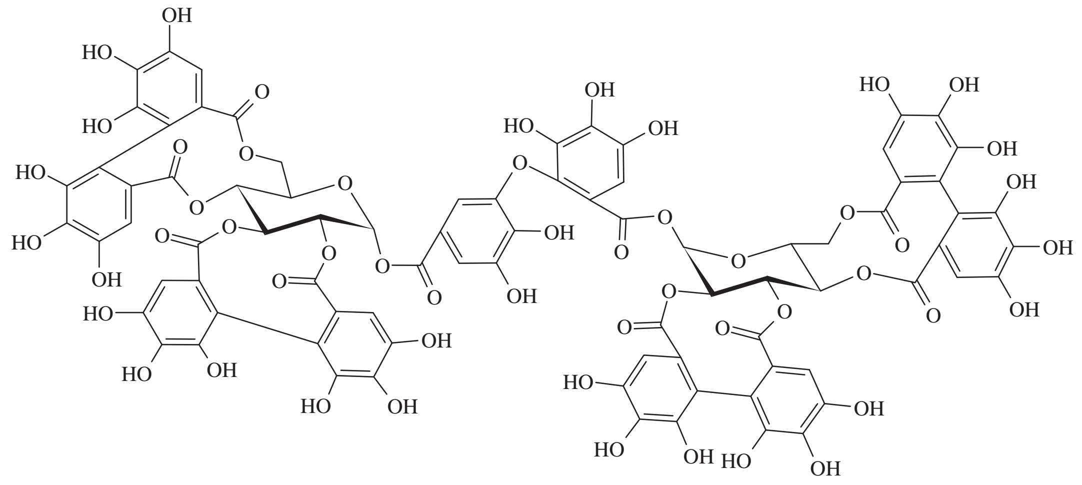
Oligomeric ellagotannins are separated according to their structure into the type GOG (where two galloyl units (G) are connected by an ether bond as in *m*-digalloyl acid, in short *m*-GOG, 1-1′), type DOG (formed by joining hexahydroxybiphenyloyl and galloyl units), type $D(OG)_2$, type *C*-glucosidic oligomers (composed of monomers joined by C–C bonds and so on. An example of a GOG oligomer is agrimoniin (**8-302**), which occurs in many plants of the Rosaceae family, such as the medicinal plant known as common tormentil (*Potentilla erecta*) and some species of agrimony (*Agrimonia* spp.). Another example is sanguiin H-6 (**8-303**) occurring in raspberries and blackberries. Examples of *C*-glycosidic oligomeric ellagotannins are grandinin and roburin A (**8-304**) found in oak wood, where they occur along with many other oligomeric ellagotannins.

8.3.6.2 Condensed tannins

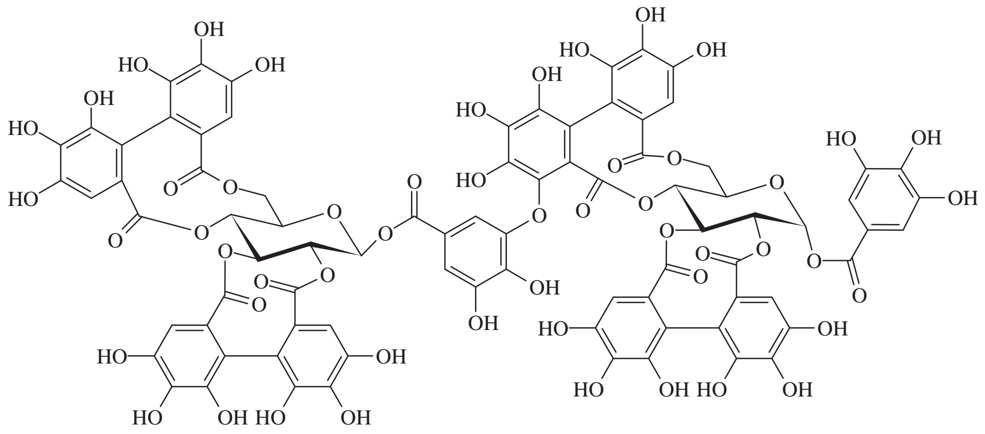
Condensed tannins called **proanthocyanidins**, and sometimes **tannoids**, are structurally diverse oligomers and polymers of flavonoid compounds with the structure of flavan-3-ol. Obsolete names are leucoanthocyanidins, leucoanthocyanins and so on (anthocyanogen, flavolanes, flavylans and flavylogens). The term leucoanthocyanidins is reserved for monomeric flavan-3,4-diols. Proanthocyanidins confer an astringent and bitter taste to fruits, fruit juices, tea, beer and many other foods and drinks. Oligomers resulting from the condensation of 2–10 basic units (the number of hydroxyl groups is also important), whose molecular weight ranges up to about 5 kDa, show this taste. Higher polymers with relative molecular weights up to of 400 kDa do not have the taste of tannins. These polymers, however, have a more important role as the pigments of red wines, and are also involved in the formation of hazes and sediments of wines, beers and fruit juices.

8.3.6.2.1 Monomers

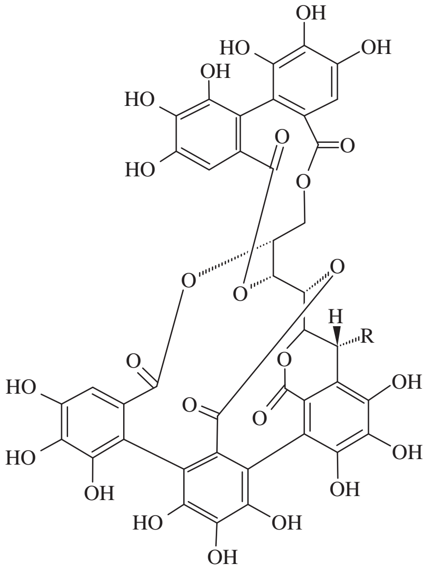
Monomeric units of condensed tannins, which lack their astringent and bitter taste, are colourless catechins, also known as 3-hydroxyflavans or flavan-3-ols (**8-305**). They are intermediates in the biosynthesis of other flavonoids. Flavan-3-ols are found in virtually all fruits, vegetables and other plant materials. Their structure depends on the stereochemistry of the flavan-3-ol units, the number of hydroxyl groups, the stereochemistry of mutual bonds of units forming oligomers, the degree of polymerisation

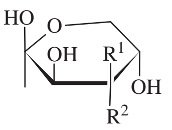


8-302, agrimoniin



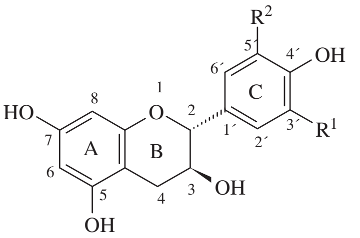
8-303, sanguiin H-6



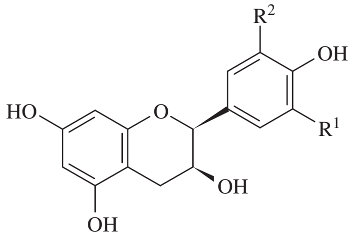
R = 

L-lyxonoyl, R^1 = H, R^2 = OH
L-lyxonoyl, R^1 = OH, R^2 = H

8-304, grandinin, R = L-lyxonyl
roburin A, R = L-xylonyl



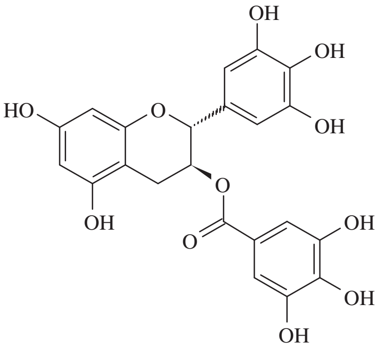
(+)-afzelechins, $R^1 = R^2 = H$
(+)-catechins, $R^1 = OH$, $R^2 = H$
(+)-gallocatechins, $R^1 = R^2 = OH$



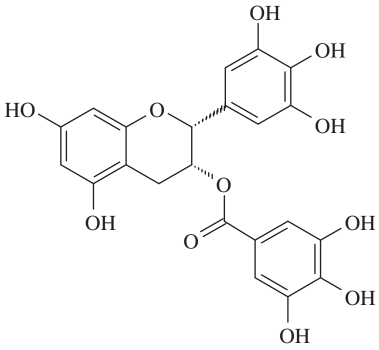
(−)-epiafzelechins, $R^1 = R^2 = H$
(−)-epicatechins, $R^1 = OH$, $R^2 = H$
(−)-epigallocatechins, $R^1 = R^2 = OH$

8-305, flavan-3-ols

and possible modification of the C-3 hydroxyl group. Monomeric flavan-3-ols with one hydroxyl group at carbon C-4′ in ring C are called afzelechins (ring B is derived from 4-hydroxybenzoic acid), monomeric flavan-3-ols with two hydroxyl at carbons C-3′ and C-4′ in ring B are called catechins (ring B is derived from protocatechuic acid) and, finally, the monomeric flavan-3-ols bearing three hydroxy groups in ring C (at C-3′, C-4′ and C-5′) are known as gallocatechins (the ring B is derived from gallic acid). Other common compounds include esters of afzelechins, catechins and gallocatechins with gallic acid bound at the C-3 position of ring C, which are called afzelechin-3-*O*-gallates, catechin-3-*O*-gallates and gallocatechin-3-*O*-gallates, respectively (**8-306** and **8-307**).



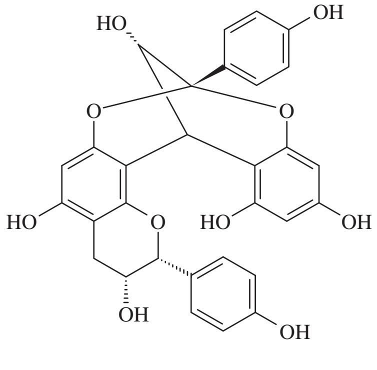
8-306, (+)-gallocatechin-3-*O*-gallate



8-307, (−)-epigallocatechin-3-*O*-gallate

Flavan-3-ols and their gallates have two chiral carbon atoms (C-2 and C-3) in the molecule and may therefore occur in four isomers. The so-called (+)-afzelechins, (+)-catechins, (+)-gallocatechins, (−)-afzelechins, (−)-catechins and (−)-gallocatechins have the C-2 and C-3 hydrogens in the (*E*)-configuration, while the corresponding epicatechins and epigallocatechins have the C-2 and C-3 hydrogen atoms in (*Z*)-configuration. In nature only (+)-afzelechins, (+)-catechins and (+)-gallocatechins that are (2*R*,3*S*)-isomers and (−)-epiafzelechins, (−)-epicatechins and (−)-epigallocatechins that are (2*R*,3*R*)-isomers are found.

(+)-Afzelechins occur abundantly in lichens and in some families of higher plants, such as in the plant families Ericaceae, Aesculaceae, Lauraceae, Rhizophoraceae and Rosaceae. For example, the bark of South African tree *Cassipourea gerrardii* (Rhizophoraceae), which has healing properties, contains afzelechin-3-*O*-α-ʟ-rhamnopyranoside and dimers of afzelechins that also rank among so-called propelargonidins. Afzelechin dimers (proanthocyanidins), such as mahuannin A (**8-308**), occur in the bark of blackthorn (*Prunus spinosa*, Rosaceae).



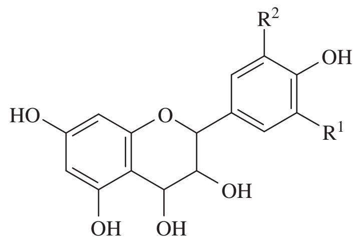
8-308, mahuannin A

The amount of catechins in fruits commonly ranges from units of mg/kg up to hundreds of units of mg/kg. For example, apples contain the major flavan-3-ols (+)-catechin (4–16 mg/kg) and (−)-epicatechin (72–103 mg/kg). Red wines, in addition to (+)-catechin (16–53 mg/l) and (−)-epicatechin (9–42 mg/kg), also contain (−)-epigallocatechin, while the level of (+)-catechin and (−)-epicatechin in white wines is significantly lower (2–6 mg/l and about 1 mg/l, respectively). Cocoa contains (−)-epicatechin,

(+)-catechin, (+)-gallocatechin and (−)-epigallocatechin, in green tea leaves the major component is (−)-epigallocatechin-3-*O*-gallate (about 60% of all catechines), (−)-epicatechin-3-*O*-gallate (10%) and in smaller amounts, as in wine and other materials, virtually all the other catechins are present.

Relatively rare compounds are flavan-3-ol glycosides. For example, (+)-catechin-7-*O*-β-D-glucoside has been isolated from several plant species, such as barley, buckwheat, vigna and rhubarb. Usually, 7-*O*-β-D-glucosides coexists in plants with other isomeric glucosides, such as 5-*O*-, 4′-*O*- and 3′-*O*-β-D-glucosides.

Monomeric flavan-3,4-diols, also known as leucoanthocyanidins (**8-309**), have two hydroxy groups in ring B at carbons C-3 and C-4. On heating in acidic solutions, they are transformed into colourless catechins and coloured anthocyanidins (aglycones of anthocyanins) from which are derived the trivial names of leucoanthocyanidins. Leucoanthocyanidin with one hydroxyl group in ring C at C-4′ is called leucopelargonidin, leucocyanidin has two hydroxyls in ring C (at C-3′ and C-4′) and leucodelphinidin has three hydroxyl groups in ring C (at C-3′, C-4′ and C-5′).



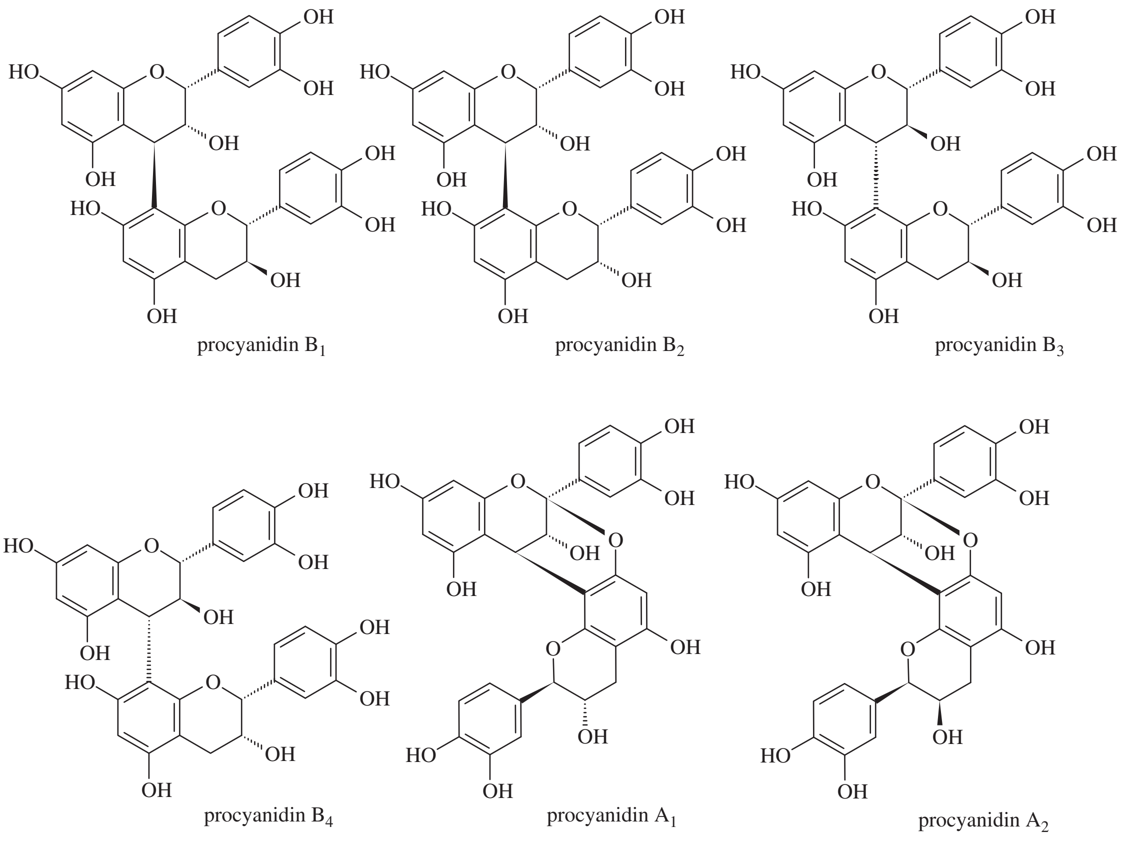
leucopelargonidin, $R^1 = R^2 = H$
leucocyanidin, $R^1 = OH$, $R^2 = H$
leucodelphinidin, $R^1 = R^2 = OH$

8-309, flavan-3,4-diols

8.3.6.2.2 Proanthocyanidins

Monomeric flavanols yield dimeric, higher oligomeric and polymeric procyanidins (proanthocyanidins) of several types. The link between the monomeric units is usually in position C-4 of the upper unit and in position C-8 of the lower unit (bond C4→C8) and can be either α- (below the ring plane) or β- (above the ring plane). These types of dimers are called procyanidins (proanthocyanidins) of type B. Flavanol units can also be linked by a bond in position C-4 of the upper unit and in position C-6 of the lower unit (bonds C4→C6). Type A proanthocyanidins have an additional ether bond between the C-7 hydroxyl of ring A of the lower unit and C-2 of the upper unit (bonds $C_2 \rightarrow O \rightarrow C_7$). Normally, the C-3 hydroxyl group of flavan-3-ol units is esterified, mostly by gallic acid. Type C proanthocyanidins are trimeric proanthocyanidins.

The most common types of dimeric proanthocyanidins have the formulae given in **8-310**. Proanthocyanidin B_1 is epicatechin-(4β→8)-catechin, proanthocyanidin B_2 is epicatechin-(4β→8)-epicatechin, proanthocyanidin B_3 is catechin-(4α→8)-catechin



8-310, common dimeric proanthocyanidins

and proanthocyanidin B_4 is catechin-(4α→8)-epicatechin. The less common proanthocyanidin B_5 is epicatechin-(4β→6)-catechin, proanthocyanidin B_6 is catechin-(4α→6)-catechin, proanthocyanidin B_7 is epicatechin-(4β→6)-epicatechin and proanthocyanidin B_8 is catechin-(4α→6)-epicatechin. Proanthocyanidin A_1 is epicatechin-(4β→8, 2β→*O*→7)-catechin and proanthocyanidin A_2 is epicatechin-(4β→8, 2β→*O*→7)-epicatechin. Examples of trimeric proanthocyanidins are proanthocyanidin C_1, epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin and proanthocyanidin C_2, catechin-(4α→8)-catechin-(4α→8)-epicatechin.

It is still not known whether the condensation of monomers proceeds by enzymatic or non-enzymatic mechanisms, or by both mechanisms. The main building units are *trans*-2,3-flavan-3-ols, that is (2*R*,3*S*)-isomers and *cis*-2,3-flavan-3-ols that are (2*R*,3*R*)-isomers. The majority of proanthocyanidins arise from (+)-catechin, which is the lower unit and (−)-epicatechin, which is the upper unit in proanthocyanidins. It is assumed that the formation of the upper units involves quinones or carbocations arising from leucoanthocyanidins, anthocyanidins and flavan-3-ols and perhaps some enzymes, such as leucoanthocyanidin reductases and polyphenol oxidases. The expected mechanism of formation of these proanthocyanidin units is outlined in Figure 8.95.

In food raw materials and foods, proanthocyanidins B are the most common types. Plants usually contain different proanthocyanidins. For example, proanthocyanidin B_1 prevails in grapes, cranberries and sorghum, proanthocyanidin B_2 in apples, cherries and cocoa beans, proanthocyanidin B_3 in strawberries and proanthocyanidin B_4 in raspberries and blueberries. In blackcurrants the dimers gallocatechin-(4α→8)-gallocatechin and gallocatechin-(4α→8)-epigallocatechin are present. Proanthocyanidins from hops consist mainly of oligomeric catechins ranging from dimers to octamers, with minor amounts of catechin oligomers containing one or two gallocatechin units. Typical compounds are procyanidin dimers (B_1, B_2, B_3 and B_4) and one trimer, epicatechin-(4β→8)-catechin-(4α→8)-catechin. Proanthocyanidins A_1 and A_2 are found, for example, in the shell of peanuts (*Arachis hypogaea*, Fabaceae), in the textured inedible rind of lychee fruit (*Litchi sinensis*, Sapindaceae) and in the bark and flowers of blackthorn (*Prunus spinosa*, Rosaceae).

Proanthocyanidins and other polyphenols are highly reactive compounds and suitable substrates for numerous enzymatic and chemical reactions. Frequently these compounds are also important components of many plant foods and often have more complex structures. For example, polymeric condensed tannins derived from procyanidin and prodelphinidin units form fibrous tissue filling the shells of hazelnuts (*Corylus avellana*, Betulaceae). The red filling of pecan shells (*Carya illinoensis*, Juglandaceae) is formed exclusively of prodelphinidin polymers. Flavan-3-ol-*C*-D-glycopyranosides, such as (−)-catechin-6-*C*-D-glucopyranoside, (−)-catechin-8-*C*-D-glucopyranoside, (−)-catechin-6-*C*,8-*C*-D-diglucopyranoside (**8-311**) and related (−)-epicatechin-derived compounds, have been shown to exhibit astringent taste and modify the bitter taste intensity of cocoa beverages. In model experiments, these conjugates arise by non-enzymatic *C*-glycosylation of flavan-3-ols by oligo- and polysaccharides.

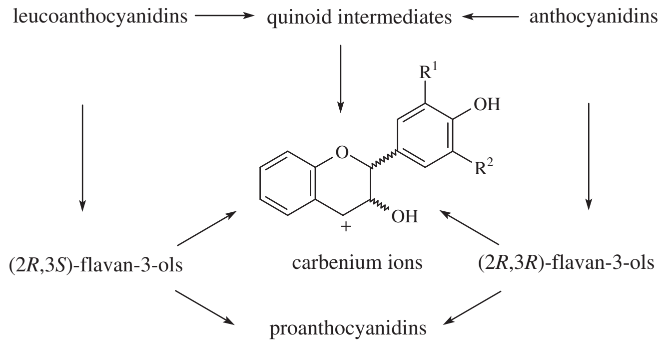
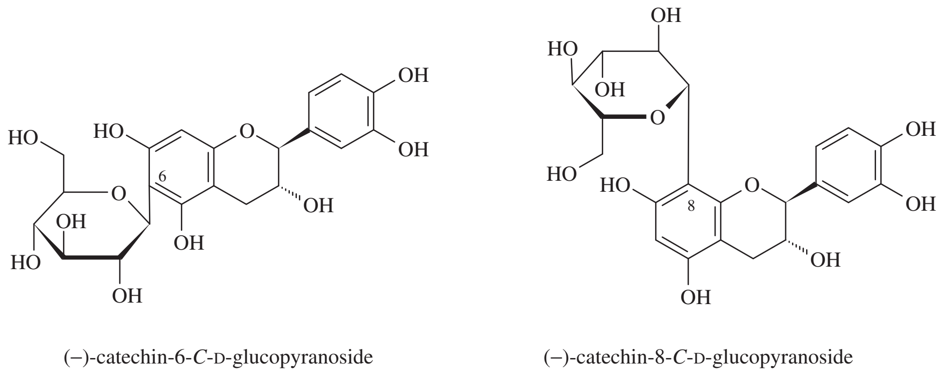


Figure 8.95 Formation of proanthocyanidins.

Grapes and wine tannins

The content of proanthocyanidins of the procyanidin type in the seeds of red grapes is 2–5 times higher than in the skin. In the seeds it is mainly oligomers with 2–6 flavanol units that are found, in the skin higher oligomers are also present. The main dimer in the skin of grapes is procyanidin B_1, which is epicatechin-(4β→8)-catechin and the major trimer is epicatechin-(4β→8)-epicatechin-(4β→8)-catechin. The seeds contain mainly procyanidin B_2, which is epicatechin-(4β→8)-epicatechin, and trimer epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (procyanidin C_1). In addition to these compounds, other dimers are found in smaller quantities,



8-311, flavan-3-ol-*C*-D-glycopyranosides

such as procyanidin B_2-3′-*O*-gallate, which is epicatechin-(4β→8)-epicatechin-3′-*O*-gallate, trimers and higher oligomers. In addition to procyanidins, prodelphinidins are also present in small amounts. Tannins of red wines have similar composition. Tannins pass to the wine mainly from the grape seeds. Their content in red wines (including monomeric catechins) is about 80–270 mg/l, while levels of tannins in white wines are much lower, at approximately 4–13 mg/l.

Catechins and lower oligomers show low astringency, but have a rather bitter taste. Oligomers containing more than four molecules of monomers are slightly bitter and very astringent. In addition to colourless tannins, red wines contain tannin complexes with polysaccharides and minerals and coloured reaction products of tannins with anthocyanins.

Black tea tannins

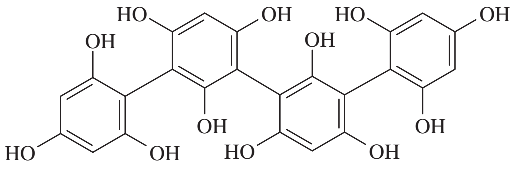
Like other plant materials, fresh tea leaves contain many catechins and proanthocyanidins, which are also found in green tea (see Section 9.12.4.1). The main catechins are epigallocatechin-3-*O*-gallate (7–13%), epigallocatechin (3–6%), epicatechin-3-*O*-gallate (3–6%), epicatechin (1–3%) and catechin (1–3% dry matter). During the fermentation of tea leaves, proanthocyanidins react with proteins, which reduce the original astringent taste of tea leaves, but enzymatic browning reactions create new astringent products.

8.3.6.3 Physiology, nutrition and use

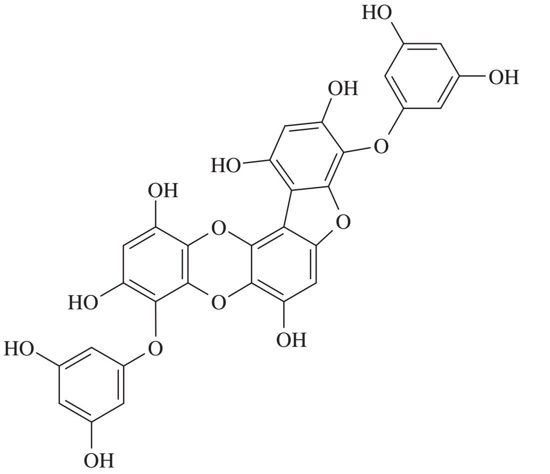
Proanthocyanidins have different physiological effects. Recently acknowledged are their anti-inflammatory and anti-allergic effects, and beneficial effects in the development of atherosclerosis (the effect is related to their reaction with free radicals). Condensed tannins have also found use as food additives. A diet that is high in tannins may, however, also show negative effects, such as lower utilisation of proteins.

8.3.6.4 Phlorotannins

In many fruits, and especially in brown algae (Phaleophyceae), the so-called **phlorotannins** occur, the basic building units of which are, instead of gallic acid, dehydrooligomers or dehydropolymers of phloroglucinols (1,3,5-trihydroxybenzenes) bound in the form of glycosides. They are integral structural components of cell walls in brown algae, but they also play a role in seaweed protection from UV radiation, in osmoprotection and defence against grazing. They may inhibit the formation of advanced glycation end products (AGEs) by scavenging reactive carbonyls. Examples of phlorotannins are tetrafucol (**8-312**) and phlorofucofuroeckol (**8-313**) from brown algae *Fucus vesiculosus* (Fucaceae) known by the common name bladder wrack.



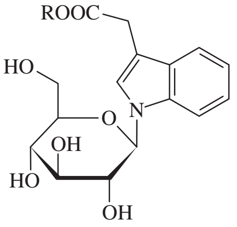
8-312, tetrafucol



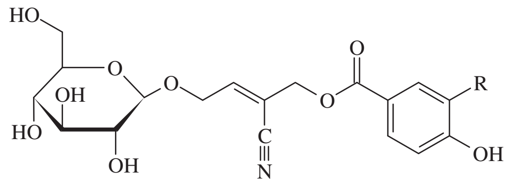
8-313, phlorofucofuroeckol

8.3.6.5 Miscellaneous astringent compounds

Astringency is traditionally thought to be induced by plant tannins in foods through tannin–protein interactions. However, a wide range of other phenolic compounds and some non-phenolic compounds in various foods can elicit astringency. Many ellagitannins that contribute to astringent properties of foods do not interact with salivary proteins and may be directly perceived through some receptors. The astringency of organic acids, such as (*Z*)- and (*E*)-isomers of aconitic acid in red currants, may be directly linked to the perception of sourness. It is mainly isoflavones (see Section 9.4.2.8) and various saponins (see Section 10.3.2.2) that contribute to the astringent taste of soya beans. The key contributors to the astringent taste of rape seeds and meals are some esters of sinapic acid called sinapines (see Section 8.2.7.1.1) and in non-fermented cocoa beans and cocoa products *N*-phenylpropenoyl amino acids (see Section 2.2.1.2.1). In addition to flavan-3-ol glycosides and aconitic acids, the key astringent and mouth-drying compounds in red currants are astringent indoles and nitriles. Examples of these compounds are 3-carboxymethylindole-1-*N*-β-D-glucopyranoside, 3-methylcarboxymethylindole-1-*N*-β-D-glucopyranoside (**8-314**), (*E*)-2-(4-hydroxybenzoyloxymethyl)-4-β-D-



8-314, 3-carboxymethyl-indole-1-*N*-β-D-glucopyranoside, R = H
3-methylcarboxymethylindole-1-*N*-β-D-glucopyranoside, R = CH_3



8-315, (*E*)-2-(4-hydroxybenzoyloxymethyl)-4-β-D-glucopyranosyloxybut-2-enenitrile, R = H
(*E*)-2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-β-D-glucopyranosyloxybut-2-enenitrile, R = CH_3

glucopyranosyloxybut-2-enenitrile derived from 4-hydroxybenzoic acid and (*E*)-2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-β-D-glucopyranosyloxybut-2-enenitrile (**8-315**) derived from vanillic acid.

8.3.7 Pungent substances

Burning, stinging, sharp, hot and pungent flavour is a characteristic phenomenon accompanying the consumption of some spices, such as hot chillies, pepper, ginger and cloves, spice mixtures that contain these spices, special sauces, some vegetables of the Brassicaceae family, such as mustard, horseradish, radish, radishes and vegetables of the Amaryllidaceae family, such as garlic and onion.

8.3.7.1 Hot pepper

The substances responsible for the hot taste of different domesticated pepper genera (*Capsicum annuum*, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum*) and their varieties are protoalkaloids **capsaicinoids** that include a group vanillylamides derived from C_8–C_{11} (*E*)-monoenic branched-chain fatty acids (isoacids and anteisoacids) and saturated fatty acids with branched or straight chains. (Z)-Isomers do not occur in peppers. About 90% of the capsaicinoids are represented, in approximately equal proportions, by capsaicin and dihydrocapsaicin, the rest are other related compounds (see Section 10.3.2.1.6). The capsaicinoids content of fresh bell (sweet) pepper cultivars (*C. annuum*) is often negligible (0.001% or less), but in some varieties of *C. annuum* (such as cayenne and jalapeño peppers) the level is in the range of 0.2–1%, and in others, as well as in some varieties of *C. frutescens* (such as tabasco and chilli peppers), it may be even higher. The hottest peppers are some varieties of *C. chinense* (such as habanero).

The taste recognition threshold of capsaicin is about 0.1 mg/kg and a concentration of 10 mg/kg causes a strong burning sensation. The burning sensation of dihydrocapsaicin is almost the same. The sharpness of both capsaicinoids is about 150–300 times higher than those of the hot components of black pepper and ginger. The pungency of hot peppers is evaluated organoleptically using Scoville heat units (SHU).[8] The taste is evaluated in a series of diluted samples (the more diluted the solution, the higher the score obtained), the last of which has no perceptible pungency. Pure capsaicin has a Scoville rating of 16 000 000, red habanero pepper of 150 000, tabasco pepper of 120 000, jalapeño pepper of 25 000 and sweet bell pepper of 0 SHU. Typical relative pungencies in SHU for the main hot pepper substances capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin are 16 000 000, 16 000 000, 9 000 000, 8 600 000 and 8 600 000, respectively.

[8]The Scoville test was invented by the American pharmacist Wilburg Lincoln Scoville in 1912. Capsaicinoids are now mainly determined by high performance liquid chromatography.

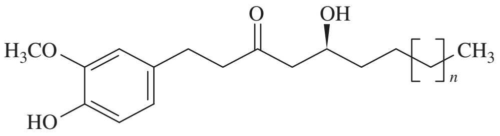
8.3.7.2 Black pepper

Black, white, green and red peppers are fruits of the same plant (*Piper nigrum*, Piperaceae) native to south India that contains protoalkaloid piperine as the major hot component; this is (2*E*,4*E*)-1-piperoylpiperidine, an amide of piperic acid, systematic name (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-*N*-piperidinylpenta-2,4-dienamide. Black pepper is obtained from immature, but already fully developed fruits that are fermented during drying. Green peppers are quickly sun-dried unripe berries. White pepper is produced from fully ripe berries by removing the red skin. The content of piperine in black and white pepper is 3–8% (see Section 10.3.2.1.2).

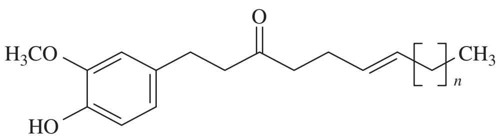
8.3.7.3 Ginger

Ginger is the rhizome of the *Zingiber officinale* (Zingiberaceae) plant native to Southeast Asia. The pungent spicy components of ginger are phenolic alkanones known as gingerols (**8-316**) and shogaols (**8-317**). Their total content in the fresh rhizome is 0.7–0.9% and 1.1–1.6% in dry rhizome.

The main and most important component is the so-called 6-gingerol, (5*S*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one, which represents about 75% of hot substances in oleoresins obtained by extraction with supercritical carbon dioxide. The

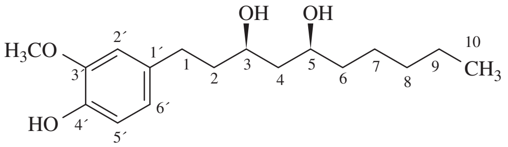


8-316, 6-gingerol, n = 2
8-gingerol, n = 4
10-gingerol, n = 6



8-317, 6-shogaol, $n = 2$
8-shogaol, $n = 4$
10-shogaol, $n = 6$

end of its side chain is derived from C_6 aldehyde (hexanal). 6-Gingerol is accompanied by its homologues 8-gingerol (11%) and 10-gingerol (15%), derived from octanal and decanal, respectively, which occur in smaller amounts in ginger rhizome. Minor pungent components of ginger are 6-shogaol (0.5%), 8-shogaol and 10-shogaol and some other related compounds are found in very small quantities, such as a homologue of 6-gingerol called 12-gingerol, its methyl derivative (methyl 6-gingerol), diketone 10-gingerdione, diol (3*S*,5*S*)-6-gingerdiol (**8-318**) and other products. 6-Gingerdiol occurs in the form of 4′-*O*- and 5-*O*-β-ᴅ-glucopyranosides. The hottest component of ginger is 6-gingerol, its homologues 8-gingerol and 10-gingerol are about 4–15 times less spicy, while 6-shogaol is 2–60 times less pungent than 6-gingerol. Ginger and its components are also known to possess such physiological features as antimicrobial, antioxidative, antitumor and antiplatelet aggregation activaties.



8-318, 6-gingerdiol

The composition of hot ginger rhizome components and ginger oleoresins vary within certain limits, depending primarily on the variety of plant and post-harvest operations. Thermally processed and stored materials, for example, contain significant quantities of shogaols that result from dehydration of gingerols. Retroaldolisation of gingerols yields pungent zingerone and the corresponding alkanals that cause an off-flavour. 6-Gingerol produces hexanal (Figure 8.96), 8-gingerol octanal and 10-gingerol decanal on degradation.

8.3.7.4 Clove

Cloves, dry flower buds of the *Eugenia caryophyllata* (Myrtaceae) tree, native to Zanzibar and other South and Southeast Asia countries, contain 15–20% of the essential oil, the main part (80–90%) of which is eugenol (4-allyl-2-methoxyphenol). Eugenol (see Section 8.2.3.1.2) has a characteristic smell of clove and a slightly pungent taste.

8.3.7.5 Cruciferous vegetables and mustard

The family of the cruciferous plants (Brassicaceae) includes a number of vegetables that have a pungent and spicy taste if eaten raw. Examples of vegetables with extreme burning taste are grated horseradish (*Armoracia rusticana*) root and certain radish varieties (*Raphanus sativus*). A less pungent taste is found in some raw vegetables of the genus *Brassica*, such as cabbage, kale and others, black (*B. nigra*), brown (also known as Indian or Chinese mustard; *B. juncea*) and white mustard seeds (*Leucosinapis album*, syn. *Sinapis alba*) that are used to produce mustard pastes. For example, the French style Dijon mustard is produced from black mustard seeds and some varieties of brown mustard, English mustard is made using white, black and brown mustard seeds (flour), and Krems mustard is made from a mixture of white and black mustard seeds.

The characteristic aroma and pungent taste of these vegetables and mustards is related to the presence of isothiocyanates. Along with other products, isothiocyanates are produced as a result of the enzyme myrosinase (thioglucoside glucohydrolase) activity in damaged plant tissues on sensory indifferent glucosinolates (see Section 10.3.2.4). One of the most common isothiocyanates is allyl isothiocyanate, which is the main pungent and spicy component of horseradish, black and brown mustard seeds and some leafy vegetables, such as cabbage, Savoy cabbage and kale.

Allyl isothiocyanate are produced from glucosinolate sinigrin, the content of which in horseradish is 27–29 g/kg (which roughly

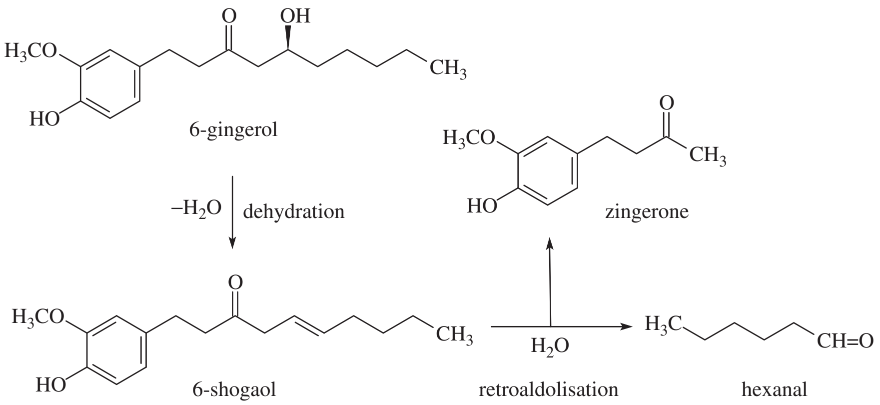


Figure 8.96 Degradation of gingerols.

corresponds to 8–9 g/kg of allyl isothiocyanate). Other isothiocyanates also contribute to the spicy flavour of horseradish, such as 2-phenylethyl isothiocyanate, which arises from glucosinolate gluconasturtiin (the content of which is within 4.2 and 7.2 g/kg), and other minority isothiocyanates. Allyl isothiocyanate is the main pungent and spicy component of Japanese horseradish, called wasabi (*Wasabia japonica*, syn. *Eutrema wasabi*), of the crucifer family (Brassicaceae). Its content in wasabi is 7–10 g/kg. In the cones of cabbage and other cruciferous vegetables, the level of sinigrin and gluconasturtiin is much lower (4–146 and 0.7-6.1 mg/kg fresh weight, respectively). In the case of cabbage, the amount of sinigrin corresponds to 1–45 mg/kg of allyl isothiocyanate.

Allyl isothiocyanate is also the main ingredient of table mustard pastes made from black mustard seeds. The level of sinigrin in seeds is 89–100% of the total glucosinolate content, which corresponds to 18–45 g/kg. The glucosinolates gluconapin (0–11%) and glucobrassicanapin (0–0.3%) are found in lower amounts. The content of allyl isothiocyanate in table mustards made from black mustard seeds is 10–18 g/kg. The brown mustard seed content of allyl isothiocyanate is only 7 g/kg. Another major isothiocyanate of brown mustard seeds is but-3-en-1-yl isothiocyanate, which is present at a concentration of 3–4 g/kg. White mustard seeds contain 4-hydroxybenzyl isothiocyanate as the main substance, which arises from glucosinolate sinalbin. 4-Hydroxybenzyl isothiocyanate is usually only mildly spicy. The sinalbin content in seeds ranges between 20 and 50 g/kg. The content of 4-hydroxybenzyl isothiocyanate in autolysed seeds is 15–37 g/kg.

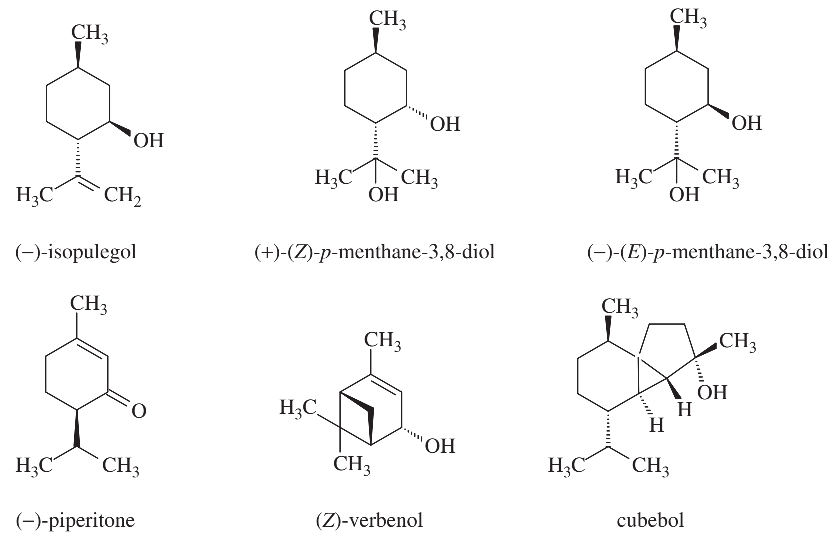
Some radish (*Raphanus sativus*) varieties, such as the grey–black radish with round roots, contain as the main pungent substance (*E*)-4-(methylthio)-but-3-en-1-yl isothiocyanate, which is produced from the glucosinolate glucoraphasatin. Its concentration in radishes is about 1–3 g/kg, resulting in an isothiocyanate content of 0.5–1 g/kg.

8.3.7.6 Alliaceous vegetables

The key flavour precursors of garlic (*Allium sativum*, Amarillidaceae), onion (*A. cepa*), shallot (*A. cepa* var. *aggregatum*), leek (*A. ampeloprasum*), chives (*A. schoenoprasum*) and other alliaceous vegetables are the sulfur-containing amino acids *S*-alk(en)ylcysteine sulfoxides also known as *S*-alk(en)ylcysteine-*S*-oxides (see Section 2.2.1.2.2). The typical aroma of these vegetables develops as a result of decomposition of *S*-alk(en)ylcysteine sulfoxides by the CS lyase called alliinase. In the intact tissue, *S*-alk(en)ylcysteine sulfoxides are located in the cytoplasm, while alliinase occurs in the vacuoles. Cutting or other damage to plants leads to rapid degradation of these amino acids to form pyruvic acid, ammonia and alk(en)yl sulfenic acids that spontaneously condense to the corresponding pungent dialk(en)ylthiosulfinates (see Section 8.2.9.1.4). These substances carry the aroma of freshly cut alliaceous vegetables, while additionally showing significant antimicrobial properties. Dialk(en)ylthiosulfinates, however, are extremely unstable substances, which quickly decompose on prolonged standing, depending on the temperature and polarity of the environment, to form a variety of secondary products (such as thiosulfonates, disulfides, trisulfides and a range of other compounds) that are responsible for the aroma of processed alliaceous vegetables.

8.3.8 Cooling substances

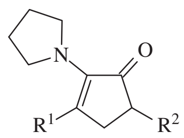
(−)-Menthol (**8-19**) is a common cooling substance used in food products. The effect of coolness evoked through stimulation of the somatosensory system can be produced by several naturally occurring molecules, mainly derived from terpenes and sesquiterpenes. Such a molecule is (−)-isopulegol (**8-319**), occurring, for example, in the essential oil of Australian lemon-scented gum (*Eucalyptus citriodora*, Myrtaceae) trees, which has 20% of the cooling



8-319, cooling-active terpenoids

power of (−)-menthol. Similar cooling agents are (+)-(*Z*)- and (−)-(*E*)-*p*-menthane-3,8-diols (**8-319**). Alternative natural cooling molecules are (−)-menthone (**8-50**) in peppermint oil, (+)- and (−)-piperitone (**8-319**) in mint oils, which are weakly cooling. Several other terpenes, such as (+)-1,8-cineole (eucalyptol, **8-31**), which occurs in essential oils of various spices and trees of the genus *Eucalyptus* and verbenol (**8-319**) from marjoram and sage oils, are slightly cooling substances. The only cooling sesquiterpene is cubebol (**8-319**), which is found in cubeb (tailed pepper, *Piper cubeba*, Piperaceae) essential oil. A considerable number of compounds showing a cooling effect have been synthesised and evaluated for the physiological sensation of cooling. Many artificial cooling compounds are menthol derivatives (esters, ethers and acetals) or have very different chemical structures.

Some cooling compounds are also present in raw food materials and foods. For example, 3-methyl- and 5-methyl-2-(1-pyrrolidinyl)cyclopent-2-en-1-one (**8-320**) were recently identified as intense cooling compounds in roasted dark malt. The cooling thresholds of these 2-aminocyclopent-2-en-1-ones in water were 29–44 and 4.5–9.0 mg/kg, respectively, while the cooling threshold of (−)-methol was 0.9–1.9 mg/kg. It was shown that the identified 2-aminocyclopent-2-en-1-ones were produced by the Maillard reaction from hexose derived cyclotene (2-hydroxy-3-methylcyclopent-2-en-1-one, see Figure 4.62) and 1-pyrrolidine formed by the Strecker degradation of L-proline.



8-320, cooling-active 1-pyrrolidinylcyclopent-2-en-1-ones
3-methyl-2-(1-pyrrolidinyl)cyclopenta-2-en-1-one, $R^1 = CH_3$, $R^2 = H$
5-methyl-2-(1-pyrrolidinyl)cyclopenta-2-en-1-one, $R^1 = H$, $R^2 = CH_3$

8.3.9 Physiology, nutrition and use

The physiological effects of hot piperidine alkaloids (see Section 10.3.2.1.2) and of capsaicinoids (10.3.2.1.7) are described elsewhere. Gingerols have only low acute toxicities, but show carminative (prevent the formation of gas in the gastrointestinal tract), antiemetic (prevents or alleviates nausea and vomiting) and antioxidative effects. Allyl isothiocyanate is used as a food flavouring agent (mayonnaise, horseradish, mustards and various special sauces), as a preservative in animal feed, in medicine as a rubefacient (counterirritant), as a fungicide, in ointments, mustard plasters, as an adjuvant (enhancing the action of medical treatment) and as a repellent for cats and dogs. The average daily intake of individuals in the United States was estimated to be less than 6 mg/day on the basis of exposure to allyl isothiocyanate in foods. Dimethyl sulfide and dimethyl disulfide are not mutagenic in mammalian cells and their acute oral toxicities are low. Physiological properties of other garlic constituents were described in Section 8.2.9.2.2. Pure sulfides, as well as allyl isothiocyanate, are irritating to the skin and the vapour irritates the eyes and nose. Eugenol is potentially hepatotoxic and an overdose may cause a wide range of symptoms. It is subject to restrictions on its use in perfumery as it may cause an allergic reaction in sensitised people.

9

Pigments and other Colorants

9.1 Introduction

The sensory quality of food and its acceptability, aside from odour, taste and texture, is generally based on colour, which is the human perception of coloured materials based on the selective absorption of light. In this chapter, the general term **pigment** is used for a substance whose presence in living cells determines their characteristic colour; in other contexts, a pigment is any material from which a dye may be prepared. The term **dye** refers to a colorant used by the industry to colour or stain various materials using dyestuffs, but not food. The general term **colorant** means any substance, such as a pigment or dye that imparts colour. The harmless substances used to impart colour to food or drink are **colour additives**.

Food pigments are usually divided into three main types:

- natural pigments
- synthetic pigments identical to natural pigments (nature identical pigments)
- synthetic pigments.

Natural pigments are coloured substances synthesised, accumulated or excreted into the environment by living cells. These pigments are:

- natural parts of foods of animal or vegetable origin due to the genetic dispositions of the given organism;
- part of other natural materials (pigments of algae, fungi, lichens or microorganisms), which are obtained in the original state as such or are structurally modified and used for food colouring as colour additives.

Coloured products derived from natural raw materials through various technological processes, such as caramel and malt extract, which contain melanoidins, are also considered natural colorants. Natural colorants additionally include copper complexes of chlorophylls and chlorophyllins, which do not occur in nature (or may be present in foods in negligible amounts), inorganic pigments, such as calcium carbonate, iron(III) oxide (iron trioxide), titanium(V) oxide (titanium dioxide) and nature identical synthetic dyes. Some natural pigments are formed only during the processing and storage of food raw materials and foods, mostly by reactions with amino acids. These adverse reactions and associated changes of natural colour are considered as discolorations and defects. The coloured products of these reactions are referred to as **reaction pigments** or **process pigments** by analogy with reaction or process flavours (process flavourings). For example, a red discoloration originating from amino acids and ascorbic acid is produced in pickled cauliflower (see Section 5.14.6.2.3), precursors of pink discoloration of onion and green to blue discoloration of garlic are amino acids and sulfenic acid resulting from (*E*)-*S*-(prop-1-en-1-yl)cysteine sulfoxide (isoalliin) (see Section 8.2.9.1.4), the precursor of the green to blue discoloration of sweet potatoes (*Ipomoea batatas*, Convolvulaceae) are amino acids and chlorogenic acid (see Section 9.12.4) and the pink and yellow discoloration in *Brassica* vegetables may be caused by reactions of amino acids with isothiocyanates (see Section 2.5.2.4).

Natural pigments are classified according to their structure, occurrence in biological materials or important properties (such as their solubility in water and fat). The following pigments are recognised according to their structure:

- Nitrogenous heterocyclic compounds, to which belong pigments derived from **pyrrole** and pigments derived from **indole**, **isoquinoline**, **pyrimidine**, respectively from **purine**, **pterine** and related **flavin**, **phenazine** and **phenoxazine**; some of these are simultaneously classified as alkaloids; the most important pigments of this group include **haem** and **chlorophyll** pigments derived from pyrrole and **melanins** and **betacyanins** derived from indole.

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- Heterocyclic compounds containing oxygen include many phenolic compounds, particularly **flavonoids**, the most important pigments of which are **anthocyanins** related to pigments derived from **isochromene** and **xanthone**.

- Other phenols and derived **quinones** include a variety of pigments, their oligomers, condensation and other products, such as **curcuminoids**.

- Terpenoids, which include in particular tetraterpenoid and some other pigments derived from tetraterpenes, known as **carotenoids**, monoterpenic pigments **iridoids** and certain other coloured compounds.

Approximately 1500 coloured compounds, also known as natural food pigments, have been isolated from foods. On the basis of their chemical structure, but also with regard to their importance, these pigments have been grouped into the following ten classes:

- tetrapyrroles (haem pigments, chlorophylls and phycobilins)

- other nitrogenous pigments (indoles and related compounds, melanins, indigoid pigments, betalains, isoquinolines, purines, pterines, isoalloxazines and phenoxazines)

- flavonoids (anthocyanins and other flavonoids, such as flavanones, flavononoles, flavons, chalcones, quinochalcones, dihydrochalcones, aurones and isoflavones)

- xanthones

- curcuminoids

- isochromenes

- quinoid pigments (quinones, such as benzoquinones, terphenylquinones, pulvic acids and troponoids, naphthoquinones, anthraquinones, including emodins, bianthrones and cochineal pigments)

- carotenoids (carotenes, xanthophylls and apocarotenoids)

- iridoids

- other terpenoids pigments.

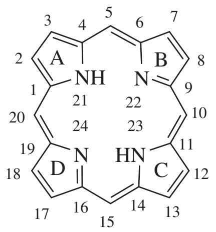
This chapter will describe in detail their occurrence in the individual food commodities, their properties, stability and important reactions, their importance in human nutrition and physiology and their use as food colourings in food technology. A section of the chapter is devoted to the so-called enzymatic browning reactions that proceed as desirable, but also as adverse reactions in many foods of plant origin. Substances used as colour additives are described in Chapter 11, which focuses on food additives. Reaction pigments are mentioned in chapters dealing with the individual nutrients.

9.2 Tetrapyrroles

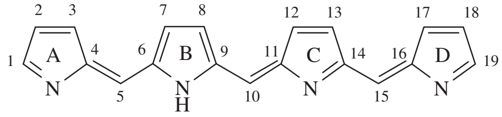
Tetrapyrrole pigments are a numerically small, but very widespread and significant group of differently coloured pigments. Their structure, a resonance-stabilised planar ring system, consists of four pyrrole rings A, B, C and D connected in a tetrapyrrole (porphyrin) circle with a conjugated double bond system through methine bridges in the α-positions of pyrrole structures (**9-1**) or arranged linearly (**9-2**). Accordingly, two basic groups of tetrapyrrole pigments are recognised:

- cyclic **porphyrins** (porphyrin pigments)

- open chain (linear) **bilins** (bilin pigments).



9-1, basic structure of porphyrin pigments



9-2, basic structure of bilin pigments

It has been reported that 28 porphyrin pigments and their precursors occur in nature. Porphyrins are chromophores of two basic groups of metalloproteins:

- pigments of animal tissues, called haem pigments or haems

- pigments of plant tissues, some algae and some microorganisms, called chlorophyll pigments or chlorophylls.

Bilins (there are six known compounds) are usually classified according to their origin as:

- phycobilins, which are pigments of some algae

- bile pigments resulting from degradation of haem pigments (pigments in urine and faeces of mammals).[1]

[1]Endogenous haem is degraded by oxidative cleavage of the porphyrin ring by the enzyme haem oxygenase inside spleen macrophages to blue–green biliverdin IX α (releasing carbon monoxide and Fe), which is reduced by biliverdin reductase to orange bilirubin IX α. Bilirubin forms a conjugate with D-glucuronic acid (diglucuronide binding to propionic acid residues). The

9.2.1 Haem pigments

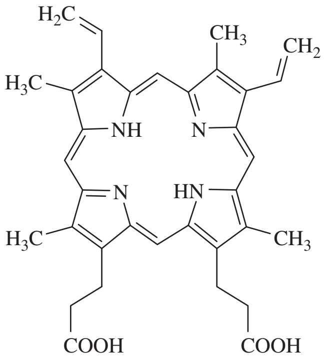
The most important haem pigments are metalloproteins:

- **myoglobin**, the red pigment of muscle tissue
- **haemoglobin**, the pigment of red blood cells (erythrocytes).

The interest in haem pigments is focused primarily on their biochemical properties in relation to the transfer of oxygen in tissues, and also to the metabolism of bile acids. However, food chemists and technologists are mainly interested in the stability and changes of haem pigments during the processing and storage of meat and meat products.

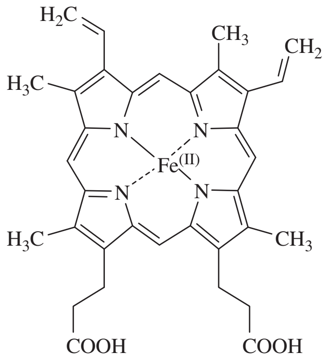
9.2.1.1 Structure and nomenclature

The basis of the structure of myoglobin, haemoglobin and derived pigments is a substituted cyclic tetrapyrrole protoporphyrin IX (**9-3**) with a central atom of divalent iron, which is called **haem** or protohaem (**9-4**). The haem molecule is almost planar. Haem is a conjugated system and although there are two coordination-covalent Fe–N bonds (the bonding electron pair apparently belongs to only one of the atoms), in fact, all four bonds with pyrrole rings are equivalent. The fifth and sixth coordination bonds of iron (above and below the plane of the planar molecule) can be occupied by different ligands. **Haemin** is protoporphyrin IX with Fe (II), where the counter ion is a chloride ion. Protoporphyrin IX with trivalent iron, an oxidation product of haem, is called **haematin**, in which a hydroxyl group is bound to the central Fe (III) atom as a counter ion.



9-3, protoporphyrin IX

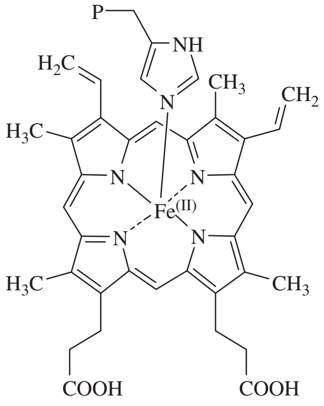
Several other haem pigments also occur in muscle tissue (such as cytochromes that carry out electron transport) containing iron in a similar porphyrin–protein complex to that in myoglobin.



9-4, haem

These pigments contribute to the colour of muscle tissue only very slightly. Haem pigments can similarly be found in the plant kingdom in the form of cytochromes and some oxidoreductases, such as catalase and peroxidase (see Section 6.2.2.7).

In myoglobin (**9-5**) and its derivatives, the fifth ligand is bound to the divalent iron atom via the imidazole group of histidine (His[93]). The fifth ligand is a protein (globin) with a relative molecular weight of 16.8 kDa. The sixth ligand is water, but this may be absent. The molecule of haemoglobin (referrd to as haemoglobin A) includes four polypeptide chains (two pairs of identical subunits α and β, for short $\alpha_2\beta_2$), each containing a single haem molecule (of relative molecular weight 64.5 kDa).



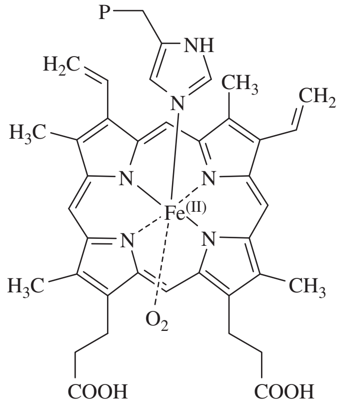
9-5, myoglobin (P = protein residue)

When myoglobin comes into contact with oxygen, it forms a reversible bright red pigment **oxymyoglobin** (**9-6**), where the sixth ligand donating both electrons to iron to form a coordinate bond is oxygen. This reaction, known as oxygenation, proceeds at oxygen partial pressures higher than 70–80 mmHg and its partial pressure in air is always higher, approximately 160 mm Hg.[2] At a very low

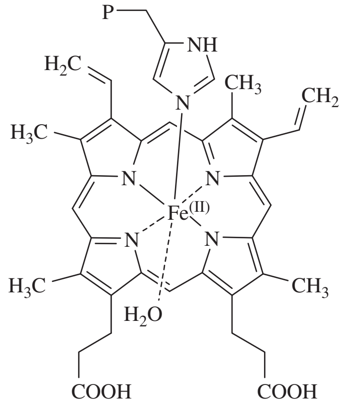
action of bacterial enzymes in the colon creates free bilirubin, which is further converted into other compounds. Of these, the most important compound is colourless urobilinogen. Part of urobilinogen gets to the bloodstream and to the kidneys, where it is oxidised to yellow urobilin IX α, which is the characteristic pigment of urine. Most urobilinogen is oxidised in the intestine to brown–red stercobilin IX α (via colourless stercobilinogen), which stains faeces.

[2]The sea level standard atmospheric pressure is 101.325 kPa, which is equal to 1 atm or 760 mmHg (torr) or 14.696 psi. So, if the air is 20.9% oxygen, the oxygen tension (partial pressure of oxygen) is 21.2 kPa, which corresponds to 159 mm Hg (1 mm Hg = 133.3 Pa).

oxygen partial pressure (<1.4 mmHg) typical of vacuum-packed meat, the electron deficiency of the iron allows it to interact ionically with water in the absence of stronger electron pair-donating ligands, which could form covalent linkages and water becomes the sixth ligand. The relevant purple–red pigment is called **deoxymyoglobin** (**9-7**). Analogously, haemoglobin gives rise to **oxyhaemoglobin** and **deoxyhaemoglobin**.

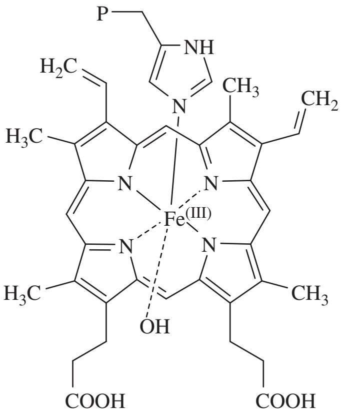


9-6, oxymyoglobin (P = protein residue)



9-7, deoxymyoglobin (P = protein residue)

An oxymyoglobin oxidation product containing trivalent iron is **metmyoglobin** (**9-8**), which is brown or gray–brown. Analogously, oxidation of haemoglobin (a loss of one electron) yields **methaemoglobin**.



9-8, metmyoglobin (P = protein residue)

Haem and its derivatives often have several different trivial names that describe the degree of oxidation of iron and bound ligands. An overview of these names is shown in Table 9.1.

9.2.1.2 Occurrence

Living organisms contain as the main pigment haemoglobin, whereas myoglobin represents only about 10% of the total iron present in the body. After slaughter and bleeding of animals, the predominant muscle pigment is myoglobin (Table 9.2). The content of myoglobin depends not only on the type of animal, but also on its age and the type of muscle (Table 9.3). The total average content of haem pigments in meat of various animals is given in Table 9.4. Dark meat (e.g. horse meat) contains more myoglobin than light meat (such as pork or veal). The meat colour is also linked to some meat defects. The levels of haem pigments in the dark and light muscle of some fish are given in Table 9.5.

Table 9.1 Nomenclature of haem pigments.

Name	Synonym	Oxidation degree Fe/other ligand	Name	Synonym	Oxidation degree Fe/other ligand
Haem	Ferroprotohaem IX	Fe(II)/H_2O	Haematin	Ferriprotohaem IX	Fe(III)/OH
	Protohaem			Hydroxyferriprotoporphyrin	
	Ferroprotoporphyrin IX			Alcaline haematin	
	Ferrous protoporphyrin IX		Haemin	Protohaemin IX	Fe(III)/Cl
	Reduced haematin			Chlorohaemin	
				Chloroferriprotoporphyrin	

Table 9.2 Haem pigments contents in beef and pork.

Meat	Myoglobin in mg/kg	Haemoglobin (mg/kg)	Proportion of myoglobin (%)
Beef	3140–7020	340–520	90–94
Pork	790–2320	360–1200	50–75

Table 9.3 Myoglobin content in beef and pork.[a]

Meat	Animal age	Muscle	Myoglobin (mg/kg)
Veal	12 days	*Longissimus dorsi*	700
Beef	3 years	*Longissimus dorsi*	4600
Pork	5 months	*Longissimus dorsi*	300
Pork	7 months	*Longissimus dorsi*	440
Pork	7 months	*Rectus femoris*	860

[a]For example, the muscle *Longissimus dorsi* is a muscle of pork loin (cutlet) and the muscle *Rectus femoris* is the main muscle of pork leg (oyster piece).

Table 9.4 Haem pigments contents in various animals.

Meat	Haem pigments (mg/kg)	Meat	Haem pigments (mg/kg)
Beef	1700–7 500	Boar	5500
Veal	438–1490	Deer	6000–7000
Pork	254–3500	Whales	9100
Horse	3620–8 000	Chicken	126–158
Lamb	2500	Turkey	125–456
Goat	6350	Goose	1586
Rabbit	200	Duck	1168

Table 9.5 Haem pigments contents in dark and light fish muscles.

Fish	Haemoglobin (mg/kg)		Myoglobin (mg/kg)	
	Dark	Light	Dark	Light
Chub mackerel (*Scomber japonicus*)	5700	100	3900	<10
Pacific saury (*Cololabis saira*)	4800	350	270	10
Pacific bluefin tuna (*Thunnus orientalis*)	4900	1700	3200	700

9.2.1.3 Use

The natural content of myoglobin is particularly important for the colour of meat and meat products. The colouring properties of pork blood haemoglobin are also employed throughout the world in some speciality products, such as blood soup, blutwurst, blood pudding, black pudding and others. The blood has to be aseptically collected from healthy pigs and swiftly processed. In China, Thailand and Vietnam, pig, chicken, duck and goose blood is used in soups and other dishes. Drinking blood from cattle is a part of the traditional diet of Maasai people.

9.2.1.4 Biochemistry, physiology and nutrition

Biosynthesis of porphyrins is based on 5-aminolaevulinic acid (5-amino-4-oxopentanoic) acid, which is synthesised from succinic acid and glycine in animal cells, and from glutamic acid in plant cells. Condensation of two molecules of 5-aminolaevulinic acid yields a straight-chain pyrrole derivative porphobilinogen that condenses to form a cyclic tetrapyrrole derivative hydroxymethylbilane, which is the precursor of uroporphyrinogen III, the common intermediate of porphyrins and corrinoids. Through a sequence of subsequent reactions, uroporphyrinogen III gives rise to protoporphyrin IX, the common intermediate of biosynthesis of haem and chlorophyll pigments, which is subsequently transformed into haemoproteins haemoglobin and myoglobin.

Haemoproteins are a large group of proteins performing different functions in animal and plant organisms. In animals, haemoglobin plays a fundamental role as a carrier of oxygen and carbon dioxide, while myoglobin is a reservoir of oxygen. Haem pigments also play a key role in energy conversion in cytochromes, and are responsible for the procuring the energy that animals produce by oxidation of nutrients in the respiratory chain and plants by photosynthesis.

As well as oxygen, myoglobin and haemoglobin can also bind other compounds. For example, poisoning by carbon monoxide (CO) transforms myoglobin into carbonylmyoglobin and haemoglobin into carbonylhaemoglobin, as the affinity of these pigments for carbon monoxide is about 130 times higher than their affinity for oxygen, therefore oxygen is displaced by the new ligand carbon monoxide. The same pigments are formed in meat packaged in a carbon monoxide atmosphere. Poisoning by cyanides leads analogously to the formation of cyanomyoglobin and cyanohaemoglobin.

From the nutritional point of view, haem pigments are of considerable importance in the supply of iron to the human organism. The so-called haem iron, which includes iron bound in myoglobin and haemoglobin and iron bound in muscle respiratory enzymes, is absorbed in the body at a level of 10–30%, while non-haem iron from only 1–5%.

9.2.1.5 Properties and reactions

The colour of meat and meat products depends on many factors, especially on the oxidation level of the central iron atom, the ligands that surround the central atom and the structure of the protein moiety.

9.2.1.5.1 *Pigments of raw meat*

Consumers usually consider the bright red colour of meat as a sign of freshness. The colour of freshly cut or minced meat comes from two types of pigments, myoglobin/oxymyoglobin and metmyoglobin. After several hours or days of exposure, oxymyoglobin can convert into metmyoglobin, which has a brown–grey colour. The colour of vacuum packed meat containing deoxymyoglobin is purplish–pink. The colour of meat packaged in an atmosphere of carbon monoxide is clearly cherry-red (typical CO concentrations range from 0.4 to 0.5%).

Atmospheric oxygen diffuses from the air into the surface layer of meat (with a thickness of up to 10 mm); myoglobin is transformed by oxygenation into bright red oxymyoglobin, and so meat acquires an attractive bright red colour (Figure 9.1). The binding of oxygen is enabled by the second histidine residue of the myoglobin molecule (His^{64}, the so-called distal histidine) through hydrogen or O–N bonds (Figure 9.2). The reaction is reversible, since oxygen dissociates from oxymyoglobin continuously. At a higher oxygen partial pressure, oxymyoglobin is fairly stable since the ligand oxygen is stabilised by a three-dimensional structure of apoprotein surrounding the haem. At low oxygen tension, however, which occurs at the interface between the oxymyoglobin and myoglobin layers during meat storage in the air, both meat pigments are slowly oxidised by air oxygen to the brown–grey, unattractive metmyoglobin, as the binding of oxygen in oxymyoglobin involves a charge migration from the haem to oxygen, probably via superoxoferrihaem $[Fe^{3+}\ O_2^{-}]^{2+}$ intermediate, which dissociates to metmyoglobin and superoxide radical (Figure 9.3).

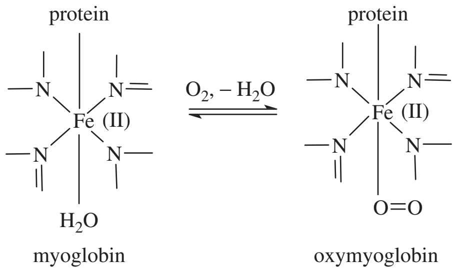


Figure 9.1 Formation of oxymyoglobin from myoglobin.

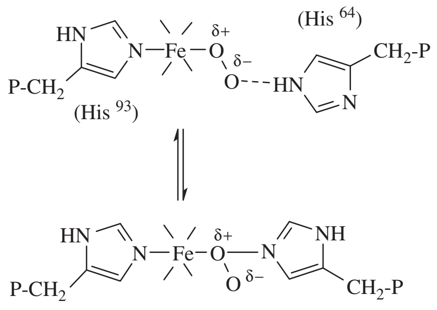


Figure 9.2 Oxygen binding and its stabilisation in myoglobin molecule (P = protein residue).

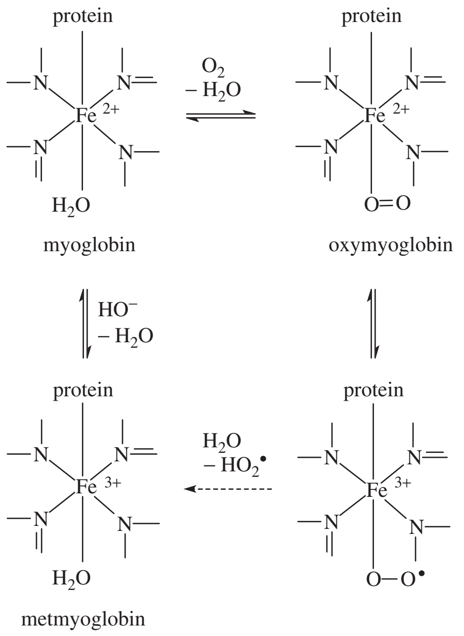


Figure 9.3 Formation of metmyoglobin in meat.

Fresh meat contains reducing agents (e.g. thiol groups of proteins and oxidoreductases with NADH as cofactors), which continuously reduce metmyoglobin to myoglobin. After oxidation of these reducing substances, a brown layer of metmyoglobin is gradually formed below the surface of the meat, and eventually the entire surface turns brown. Browning is an indication that the meat is not particularly fresh. The autoxidation of haemoglobin to methaemoglobin occurs in the same way. The colour of vacuum-packed meat, and of meat packaged in a carbon monoxide atmosphere, is more stable.

The oxidation of Fe^{2+} to Fe^{3+} is accelerated by light and at higher temperatures and lower pH. For example, the oxidation is faster after *rigor mortis* dissipates (when the pH of the meat is 5–6), than immediately post-mortem, when the pH of the meat is almost neutral. Oxidation is also accelerated by certain metal ions, especially Cu^{2+} ions; other metals, such as Fe^{3+}, Zn^{2+} and Al^{3+} are less active.

In addition to the change in meat colour, autoxidation of meat pigments has other undesirable consequences. Oxygen bound in oxymyoglobin dissociates after protonation, and autoxidation of oxymyoglobin to metmyoglobin produces free superoxide radicals (Figure 9.3). The distal histidine acts as a proton donor, as do certain protein functional groups of the environment. Superoxide free radicals and the eventually formed hydroxyl radicals can initiate autoxidation of fatty acids, which can lead to rancidity in fatty parts of the meat. In all organisms *in vivo* (except bacteria) the superoxide radical is removed by the action of superoxide dismutase, which catalyses the dismutation of superoxide radical into oxygen and hydrogen peroxide. Hydrogen peroxide is then decomposed to water and oxygen by catalase. In meat, superoxide radical rapidly dismutates to oxygen and hydrogen peroxide.

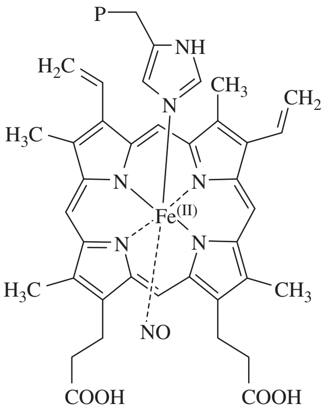
9.2.1.5.2 Meat colour stabilisation

Salt has been used in the practice of meat preserving for centuries because of its ability to draw out moisture and prevent microbial growth. Long before refrigeration and freezing, salt was the preservative of choice for ancient civilisations, as well as sailors and early pioneers. Salt preservation is still a valid method that is widely used. The prevention of changes in colour during heat treatment and simultaneous inhibition of the growth of bacterial pathogenesis is achieved by the addition of curing salts. Curing salts, usually containing salt (sodium chloride) in a mixture with either nitrites or nitrates, are often used in combination with other features, such as smoking. In most cured meat products, the addition of nitrites and/or nitrates is necessary to prevent the growth and toxin production by the bacteria *Clostridium botulinum*. Nitrates have no direct activity against *C. botulinum*, but they act as reservoirs of nitrites that are generated from nitrates by microbial activity. The use of nitrates and nitrites in cured meat and meat products must comply with the EU legislation.[3]

The reaction of meat pigments with nitrites and/or nitrates, which provides the cured meat colour and flavour, is very complex. Typically, the product is cured by injection with the curing salt solution, followed by immersion in brine, or simply immersion only is used. The dry curing process involves the dry application of the curing mixture containing nitrites and/or nitrates, salt and other components to the surface of the meat, followed by a period of stabilisation/maturation. Nitrites are first reduced to nitrogen monoxide (nitric oxide) by myoglobin and other reducing agents, while myoglobin is oxidised to metmyoglobin:

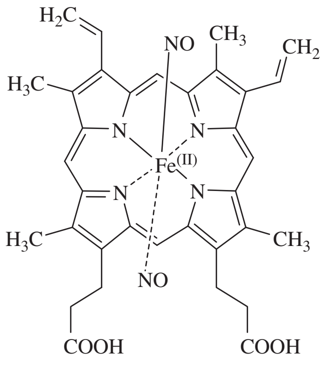
$$\text{myoglobin} + NO_2^- \rightarrow \text{metmyoglobin} + NO$$

The resulting nitrogen monoxide reacts with another molecule of myoglobin to form a red pigment **nitrosomyoglobin** (formerly also called nitroxymyoglobin or nitrosylmyoglobin, **9-9**). The reaction of nitrogen monoxide with metmyoglobin yields nitrosometmyoglobin that can also be formed by reaction of nitrogen monoxide with metmyoglobin nitrite (**9-8**, instead of N=O is the NO_2^- anion is present). Nitrosomyoglobin also occurs in meat, which does not contain nitrites, when smoking meat in the home, as flue gas contains various nitrogen oxides. Metmyoglobin formed by oxidation of myoglobin is reduced back to myoglobin either chemically (the electron donors are thiol groups of proteins and other thiols) or by the system of dehydrogenases containing NAD as a cofactor. In the same way nitrosometmyoglobin is also reduced to form nitrosomyoglobin. The reaction of nitrites with oxymyoglobin in the surface layers of meat leads to oxidation of nitrite, and even of haem iron (the oxidising agent is oxygen bound in oxymyoglobin). This reaction explains the grey–brown colour of the surface layers of cured meat, while meat is pink inside.



9-9, nitrosomyoglobin (P = protein residue)

Nitroxymyoglobin is a very stable pigment at normal temperatures as the bound ligand nitrogen monoxide is stabilised, as oxygen in oxymyoglobin, by the N–N bond with the distal histidine (His[64]). During heat treatment, nitroxymyoglobin is denatured, splits off the globin and the vacant coordination bond of iron is occupied by another nitric oxide molecule, which yields the most important pigment of heat-treated cured meat and meat products with NO ligands in both axial positions that is called **nitrosohaemochrome** (also nitroxyhaemochrome and nitrosylmyochromogen, **9-10**). At the same time other meat proteins denature, and their accessible thiol groups then reduce the metmyoglobin that are produced back to myoglobin, which can then be used for the formation of a further portion of nitrosohaemochrome.



9-10, nitrosohaemochrome

Better colour of smoked meat products is achieved by adding reducing agents together with nitrites. The most commonly used agents are L-ascorbic acid, sodium L-ascorbate, L-ascorbyl

[3]The use of nitrates and nitrites in cured meat products must comply with the provisions set out in Directive 2006/52/EC, which amends Directive 95/2/EC on additives other than colours and sweeteners. Currently authorised as food additives are sodium and potassium nitrites and sodium and potassium nitrates that may be sold only in a mixture with salt or a salt substitute. The indicative ingoing amount of potassium and sodium nitrites that are authorised for use are 150 mg/kg and the residual amount is 50 mg/kg (KNO_2) in non-heat-treated, dried meat products, 100 mg/kg ($NaNO_2$) in other cured meat products, canned meat products and 175 mg/kg ($NaNO_2$) in cured bacon. The indicative ingoing amount of potassium and sodium nitrates is 300 mg/kg in all cured products and the residual amount in cured and canned meat products is 250 mg/kg, in pickled herring and sprat 200 mg/kg and in hard, semi-hard and semi-soft cheeses and dairy-based cheese analogues 50 mg/kg.

palmitate or sodium L-isoascorbate (erythorbate). The addition of ascorbate is preferred because it does not impair the pH of the meat. Reducing agents reduce metmyoglobin back to myoglobin and also produce nitrogen monoxide from nitrites without the participation of myoglobin (H_2A = ascorbic acid, A = dehydroascorbic acid):

$$2\ \text{metmyoglobin} + H_2A \rightarrow 2\ \text{myoglobin} + A$$

$$2NO_2^- + H_2A + 2\ H^+ \rightarrow 2NO + A + 2H_2O$$

If nitrates are used instead of nitrites, they are reduced to nitrites by the microflora that are present. If ascorbic acid is used, it reduces nitrates to nitrites as follows:

$$2NO_3^- + H_2A + 2H^+ \rightarrow 2NO_2^- + A + 2H_2O$$

If protected from light, nitrosohaemochrome is stable to oxidation even at elevated temperatures, but in light the molecule dissociates to nitrogen monoxide, which is oxidised to nitrogen dioxide, and the haem produced is rapidly oxidised to haematin or further degraded in the presence of oxygen or other oxidising agents. Meat products then become greyish and fade.

Myoglobin can degrade through a number of pathways producing dark brown, yellow and even green pigments that may cause colour defects, for example, in minced meat and some meat products. The colour change can be also caused by air oxygen, fatty acid hydroperoxides or hydrogen peroxide generated, for example, from added ascorbic acid or by lactobacilli in fermented salami. The oxidation occurs even when nitrites or ascorbic acid are over-dosed and in the absence of reducing substances in meat, which leads to grey and green pigments.

Myoglobin readily reacts with hydrogen peroxide and fatty acid hydroperoxides. The oxidation of myoglobin by hydrogen peroxide starts with the formation of a radical in the globin portion of the molecule and conversion of the haem Fe^{3+} iron into the Fe^{4+} state. The reaction probably proceeds via a red radical intermediate **perferrylmyoglobin** (containing tetravalent iron), which rapidly breaks down to green **ferrylmyoglobin** (containing tetravalent iron, native globin and oxygen atom as the ligand):

$$\text{myoglobin } (Fe^{2+}–O_2) + H_2O_2 \rightarrow \text{perferrylmyoglobin}^\bullet$$
$$\rightarrow \text{ferrylmyoglobin } (Fe^{4+}–O_2) + H_2O + O_2^-$$

Hydrogen peroxide can also irreversibly oxidise the porphyrin ring in myoglobin, yielding green **choleglobin** (containing native globin, Fe^{3+} or Fe^{2+} and a covalently or ionically bound hydroperoxide O–OH group as the ligand). Choleglobin is rapidly degraded to yield globin, iron and a tetrapyrrole.

In the presence of sulfhydryl substances, myoglobin can be reversibly reduced at one double bond by the reaction with sulfane and oxygen (via ferrylmyoglobin) to green **sulfmyoglobin** containing native globin, Fe^{2+} and covalently bound SH group as the ligand:

$$\text{ferrylmyoglobin } (Fe^{4+}–O_2) + HS^-$$
$$\rightarrow \text{sulfmyoglobin } (Fe^{2+}–SH) + HO^-$$

Oxidation of sulfmyoglobin (loss of one electron) yields red metsulfmyoglobin containing Fe^{3+} and an ionically bound SH group as the ligand. During heat treatment, the globin of green pigments is denatured and split off with the formation of green **verdochrome** (containing Fe^{3+} and ionically bound water as the ligand) with porphyrin ring opening.

9.2.1.5.3 Pigments of heat-processed meat

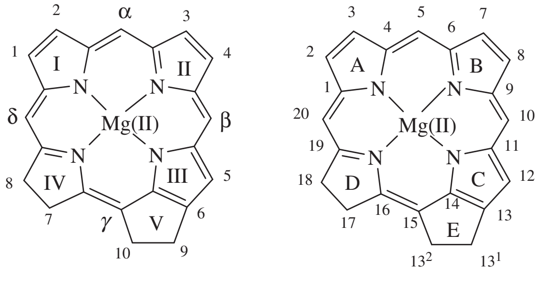
When meat is cooked, globin denatures at temperatures above about 65 °C (along with other proteins) to a degree that depends on pH and the intensity and time of thermal processing, but the haem portion remains intact. The colour of the haem pigments containing denatured globin is determined by the oxidation state of haem iron and the sixth ligand molecule, which is attached to haem. The denatured globin cannot actually maintain iron in the reduced state, therefore haem is oxidised to haematin (metmyoglobin with denatured globin) and the red meat colour is changed to brown and then even further to grey–brown.

9.2.2 Chlorophylls

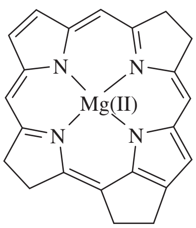
Chlorophyll pigments or **chlorophylls** are a group of green pigments, which are found in tissues of organisms providing photosynthesis. Originally the name chlorophylls were only used for green pigments involved in photosynthesis in higher plants, but later it was extended to all photosynthetic porphyrin pigments that occur in almost all higher plants, mosses and algae. Some bacteria also contain pigments known as **bacteriochlorophylls**. Several different types of chlorophylls have been described and it is assumed that other pigments may yet be found in the lesser-known species of algae.

9.2.2.1 Structure and nomenclature

The structural base of most chlorophylls is the cyclic tetrapyrrole **17,18-dihydroporphyrin** (**9-11**) derived from protoporphyrin IX. Unlike haem pigments, chlorophylls have a partially reduced ring D (they are 17,18-dihydroporphyrins), another ring E generated by cyclisation of the propionic acid residue in position C-13 and as the central atom they have chelated magnesium (magnesium ion of oxidation number +2). Bacteriochlorophylls are **7,8,17,18-tetrahydroporphyrins** (**9-12**).



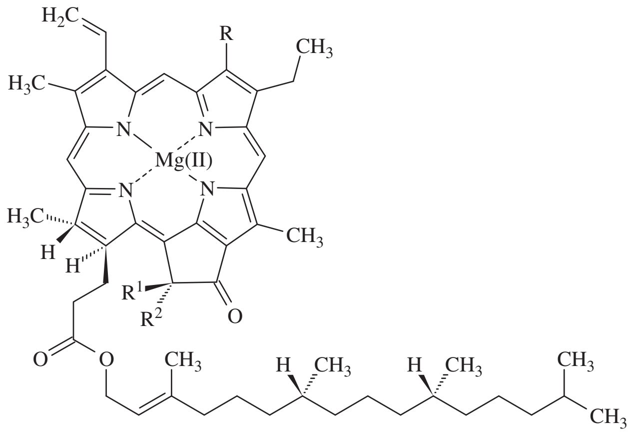
9-11, basic structure of dihydroporphyrin pigments by the older Fischer system (left) and the IUPAC recommended system (right)



9-12, basic structure of tetrahydroporphyrin pigments

From the food perspective, the most important chlorophylls are chlorophyll *a* and chlorophyll *b* and their degradation products **phaeophytins**, respectively, 17,18-dihydrophaeophytins (phaeophytin *a* and phaeophytin *b*). Some other products of chlorophyll biosynthesis and degradation are also components of foods, such as **chlorophylides**, respectively, 17,18-dihydrophaeophorbideato-Mg(II) (chlorophylide *a* and chlorophylide *b*), and **phaeophorbides**, respectively, 17,18-dihydrophaeophorbides (phaeophorbide *a* and phaeophorbide *b*).

Chlorophyll *a*, 17,18 dihydrophaeophytinato-Mg(II), is 17,18-dihydroporphyrin substituted in positions C-2, C-7, C-12 and C-18 by methyl groups, in position C-3 by a vinyl group and in position C-8 by an ethyl group. In position C-17 there is a residue of propionic acid esterified by diterpenic alcohol phytol, (2*E*,7*R*,11*R*)-3,7,11,15-tetramethylhexadec-2-en-1-ol, which constitutes a lipophilic side chain of the chlorophylls. In position C-13^1 of ring E there is an oxo group and in position C-13^2 a carboxymethyl group (**9-13**). Chlorophylls *a* and *b* differ only by the substituent at C-7: a methyl group in chlorophyll *a* and a formyl group in chlorophyll *b*. Chlorophylls *a*′ and *b*′, which differ in their configuration of C-13^2 substituents, arise by epimerisation of chlorophyls *a* and *b* in thermal processes and, particularly, in alkaline media. The structures of all currently known chlorophylls and bacteriochlorophylls are listed in Table 9.6.



9-13, chlorophylls

chlorophyll *a*, R = CH_3, R^1 = H, R^2 = $COOCH_3$
chlorophyll *b*, R = CH=O, R^1 = H, R^2 = $COOCH_3$
chlorophyll *a*´, R = CH_3, R^1 = $COOCH_3$, R^2 = H
chlorophyll *b*´, R = CH=O, R^1 =$COOCH_3$, R^2 = H

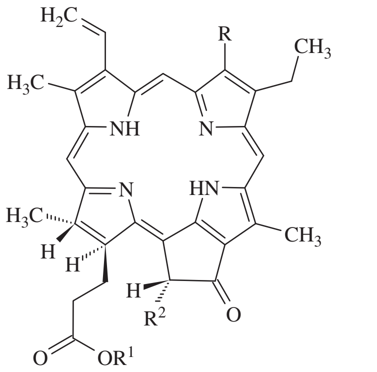
Phaeophytins are derived from chlorophylls, with hydrogen replacing magnesium (**9-14**). Hydrolysis of phytol in chlorophylls

Table 9.6 Overview of chlorophyll and bacteriochlorophyll structures.

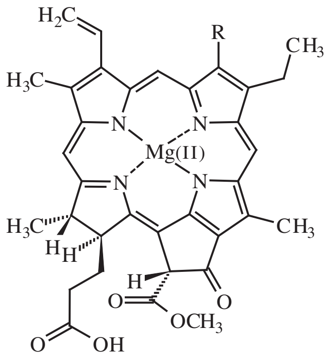
Pigment	Substituent at carbon[a]					
	C-3	C-7	C-8	C-17	C-18	C-20
Dihydroporphyrins						
Chlorophyll *a*	CH=CH_2	CH_3	CH_2CH_3	Phytyl and H	CH_3 and H	
Chlorophyll *b*	CH=CH_2	CH=O	CH_2CH_3	Phytyl and H	CH_3 and H	
Chlorophyll *d*	CH=O	CH_3	CH_2CH_3	Phytyl and H	CH_3 and H	
Tetrahydroporphyrins						
Bacteriochlorophyll *a*	(C=O)CH_3	CH_3 a H	CH_2CH_3 a H	Phytyl and H	CH_3 and H	
Bacteriochlorophyll *b*	(C=O)CH_3	CH_3 a H	CH_2CH_3 a H	Farnesyl and H	CH_3 and H	
Bacteriochlorophyll *c*	CH(OH)CH_3	CH_3 a H	CH_2CH_3 a H	Farnesyl and H	CH_3 and H	CH_3 or CH_2CH_3
Bacteriochlorophyll *d*	CH(OH)CH_3	CH_3 a H	CH_2CH_3 a H	Farnesyl and H	CH_3 and H	
Bacteriochlorophyll *e*	CH(OH)CH_3	CH=O a H	CH_2CH_3 a H		CH_3 and H	CH_3 or CH_2CH_3
Porphyrins						
Chlorophyll c_1	CH=CH_2	CH_3	CH_2CH_3	CH=CHCOOH	CH_3	
Chlorophyll c_2	CH=CH_2	CH_3	CH=CH_2	CH=CHCOOH	CH_3	

[a]The substituent in positions C-2, C-12, C-18 is always CH_3 group (with the exception of bacteriochlorophyll e, where in the position C-12 is CH_2CH_3 group), in positions C-13 and C-15 is the rest of cyclopentanone, other positions are not substituted.

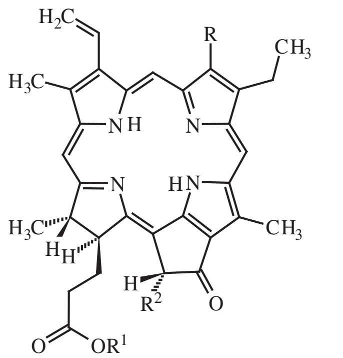
yields chlorophylides (**9-15**). Replacing the magnesium ion by two hydrogen ions in chlorophylides gives rise to phaeophorbides (**9-16**). Replacing phytol (phytyl residue) in phaeophorbides with methanol (methyl group) gives methylphaeophorbides (**9-16**). Substitution of the carboxymethyl group on C-13^2 with hydrogen produces pyrophaeophytins (**9-14**) from phaeophytins and phaeophorbides from pyrophaeophorbides (**9-16**).



9-14, phaeophytins and pyrophaeophytins
phaeophytin *a*, R = CH_3, R^1 = fytyl, R^2 = $COOCH_3$
phaeophytin *b*, R = CH=O, R^1 = fytyl, R^2 = $COOCH_3$
pyrophaeophytin *a*, R = CH_3, R^1 = H, R^2 = H
pyrophaeophytin *b*, R = CH=O, R^1 = H, R^2 = H



9-15, chlorophylides
chlorophylide *a*, R = CH_3
chlorophylide *b*, R = CH=O



9-16, phaeophorbides, methylphaeophorbides and pyrophaeophorbides
phaeophorbide *a*, R = CH_3, R^1 = H, R^2 = $COOCH_3$
phaeophorbide *b*, R = CH=O, R^1 = H, R^2 = $COOCH_3$
methylphaeophorbide *a*, R = CH_3, R^1= CH_3, R^2 = $COOCH_3$
methylphaeophorbide *b*, R = CH = O, R^1= CH_3, R^2 = $COOCH_3$
pyrophaeophorbide *a*, R = CH_3, R^1 = H, R^2 = H
pyrophaeophorbide *b*, R = CH = O, R^1 = H, R^2 = H

Table 9.7 Occurrence of chlorophylls and bacteriochlorophylls.

Pigment	Organism
Chlorophyll *a*	All photosynthetic organisms, higher plants, algae (*Cyanophyta*, *Prochlorophyta*), bacteria
Chlorophyll *b*	Higher plants, algae (*Chlorophyta*, *Euglenophyta, Prochlorophyta*), bacteria
Chlorophyll *c*	Algae (*Phaeophyta*, *Pyrrophyta*, *Bacillariophyta*, *Chrysophyta*, *Prasinophyta*, *Cryptophyta*, *Xanthophyta*)
Chlorophyll *d*	Algae (*Rhodophyta, Chrysophyta*)
Bacteriochlorophyll *a*, *b*	Bacteria (*Chromatiaceae*, *Rhodospirillaceae*)
Bacteriochlorophyll *c*, *d*, *e*	Bacteria (*Chlorobiaceae*, *Chloroflexaceae*)

9.2.2.2 Occurrence

Chlorophylls are found in all green plants, algae and also in some microorganisms (Table 9.7). Germ cells of flowering plants (angiosperms, Angiospermae) that grow in the dark lack chlorophyll, because the enzyme protochlorophylide reductase (which reduces the colourless intermediate protochlorophylide to chlorophylide) requires light. These so-called etiolated plants will start to synthesise chlorophyll after exposure to light. Anoxygenic photosynthetic bacteria do not need light to catalyse this reaction, as they contain other enzymes. Cyanobacteria, algae, lower plants and gymnosperms (Gymnospermae), such as conifers, synthesise chlorophyll even in the dark.

Food contains mainly chlorophyll *a* and chlorophyll *b* originating from higher plants. These two forms are present in the ratio of about 3 : 1. The total chlorophyll contents in the green parts of plants are very different. Grapes, for example, only contain chlorophyll at a level of about 6 mg/kg, whereas spinach contains up to 790 mg/kg of chlorophyll, while the ratio of concentrations of both compounds ranges from 2.4 to 3.1.

Chlorophylls in living cells are located in chloroplasts (the thylakoid membrane, which is a lipid double layer consisting mainly of galactolipids and a small amount of membrane phospholipids containing higher amounts of linoleic and linolenic acids). Chlorophylls in chloroplasts are associated by non-bonding interactions with proteins with a relative molecular weight of 26–28 kDa. These complexes are further associated along the phytol chain

with carotenes, xanthophylls and tocopherols. Ten molecules of chlorophylls are associated with about one lipophilic molecule.

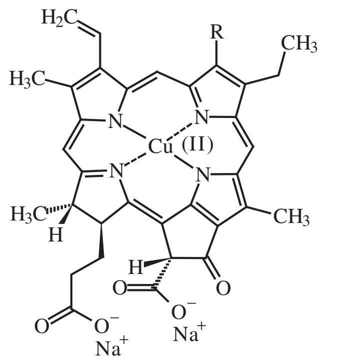
9.2.2.3 Use

Chlorophylls are practically the only natural green pigments that occur naturally in almost unlimited quantities. The total amount of chlorophylls that are produced annually on Earth is estimated at 11.5×10^8 tons, of which 2.9×10^8 tons arise on land and 8.6×10^8 tons in the oceans. They mainly occur as natural food pigments, because their great instability prevents further utilisation. For example, chlorophylls *a* and *b* are not used as such (underivatised), since their acquisition from natural resources is costly and the product is not stable enough.

The most common forms of green pigments used as food additives are fat-soluble mixtures of green pigments, commercially known as chlorophylls or chlorophyll copper complexes. Chlorophylls are mixtures of products consisting mainly of phaeophytins *a* and *b* and their epimers (known as phaeophytins *a′*and *b′*) differing in the arrangement of position C-13^2. Most of the chlorophylls for food use are obtained from edible plants growing on land, mainly nettles, alfalfa, parsley leaves and others. In Japan, chlorophylls are extracted from green silkworm (*Bombyx mori*) excrements. A promising procedure is the isolation of chlorophylls from unicellular phytoplankton.

The water-soluble greyish-green semi-synthetic mixture of pigments called **chlorophyllins** (or phytochlorins) is prepared by alkaline hydrolysis of chlorophylls, which results in hydrolysis of methyl and phytyl esters. Chlorophyllins contain a central magnesium atom and sodium or potassium salts of various products resulting from chlorophylls after alkaline hydrolysis, such as salts of phaeophorbides *a* and *b*, salts of phaeophytins *a* and *b* and their *allo*-forms (diastereoisomers), pigments with a carboxyl group at position C-13 and a carboxyethyl group at position C-17 and products with an opened cyclopentanone E ring that contains a newly formed carboxymethyl group in position C-15. Other products may include compounds with an oxidised ring D not connected to ring E, and possibly a reduced vinyl group in position C-3. These products were previously known, for example, as chlorins, purpurins and rhodins. Typically chlorin e_6 is a phaeophorbide derived product (it does not contain a magnesium ion or bound phytol) with a carboxyl group at position C-13, a carboxyethyl group at position C-17 and a carboxymethyl group in position C-15. Instead of chlorophyllin, the recommended name is now rhodochlorin. Stable bright green pigments with the trade name **chlorophyllin copper complex** are obtained by acidification of chlorophyllins in the presence of copper salts. Instead of magnesium, these products contain Cu(II) as the central atom. An example of a trisodium salt of chlorophyllin copper complex is given in formula **9-17**.

Chlorophylls are used as food pigments, for example, for dyeing pasta products, beverages, sweets, soups, frozen yoghurts and creams. These dyes are used most heavily in cosmetic products. The use of a chlorophyllin copper complex (under the name of natural green 3) is usually limited to certain products, such as chewing gum. These pigments have also found use in alternative medicine, such as odour control of wounds, injuries and radiation burns and treatment of calcium oxalate kidney stones.



9-17, trisodium chlorophyllin copper complex

9.2.2.4 Biochemistry, physiology and nutrition

The common intermediate of haem and chlorophyll pigments biosynthesis, protoporphyrin IX, is transformed into chlorophylide by a sequence of several reaction steps. Chlorophyll *a* arises from chlorophylide in a reaction with phytyl diphosphate that is produced by the reduction of geranylgeranyl diphosphate. Oxidation of the C-7 methyl group in chlorophyll *a* to a formyl group yields chlorophyll *b*.

Chlorophylls, as catalysts of photosynthesis, are normal constituents of foods of plant origin, and are ingested daily over a lifetime by both animals and humans without any appreciable health risks. In people with certain genetic abnormalities such as albinism, also called achromia (a disorder characterised by the complete or partial absence of pigment in the skin, hair and eyes, due to the absence or defect of tyrosinase producing pigments called melanins), inflammation of the skin (dermatitis) is observed after exposure to sunlight. Photosensitivity is associated with the presence of chlorophylides, particularly phaeophorbides, methylphaeophorbides and hydroxyphaeophorbides (having a hydroxyl group on C-13^2 instead of H atom), which are found in certain foods and food supplements (such as products containing algae of the genus *Chlorella*).

9.2.2.5 Properties and reactions

Chlorophylls and phaeophytins are fat-soluble pigments. Chlorophylides and phaeophorbides are hydrophilic pigments soluble in water due to the absence of phytol. The colours of chlorophylls and derived products are given in Table 9.8.

An overview of the main reactions of chlorophylls and their degradation products is shown in Figure 9.4. The most important reaction occurring during food processing is the replacement of the central magnesium atom by hydrogen and the formation of phaeophytins. This reaction, called **phaeophytinisation**, proceeds even in weakly acidic media, which are normally found in fruits and vegetables, such as sweet pickled greengages and sour pickled cucumbers. The action of the enzyme chlorophyllase or strong acids

Table 9.8 Colour of chlorophylls and some of their degradation products.

Pigment	Colour	Pigment	Colour
Chlorophyl *a*	Blue-green	Bacteriochlorophyll *c*	Green
Chlorophyl *b*	Green	Bacteriochlorophyll *d*	Green
Chlorophyl c_1	Yellow-green	Bacteriochlorophyll *e*	Green
Chlorophyl c_2	Yellow-green	Phaeophitin *a* and *b*	Olive brown
Chlorophyl *d*	Blue-green	Chlorophylide *a* and *b*	Green
Bacteriochlorophyll *a*	Gray-pink	Phaeophorbide *a* and *b*	Olive brown
Bacteriochlorophyll *b*	Brown-pink	Open ring products	Colourless

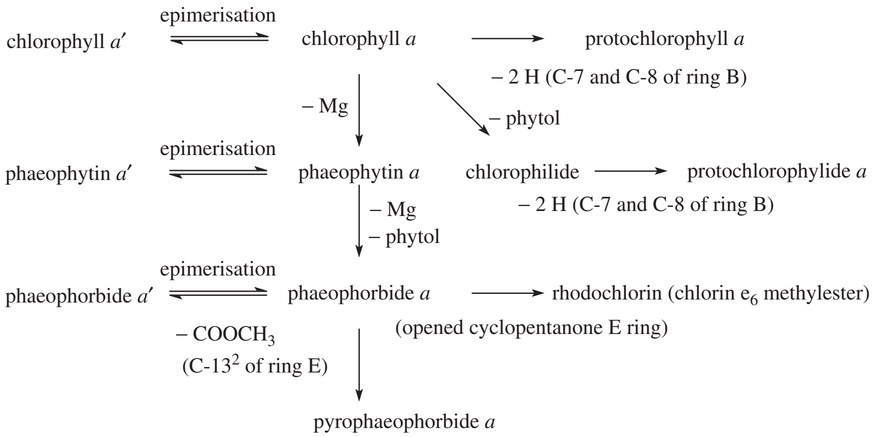
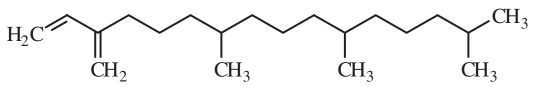


Figure 9.4 Main reactions of chlorophylls during food processing.

results in phytol hydrolysis in phaeophytins and the formation of phaeophorbides. In enzymatically active plant tissues, the hydrolysis of phytol in chlorophylls by chlorophyllase yields chlorophylides. Replacing magnesium with hydrogen in chlorophylides in weakly acidic media leads to phaeophorbides, which are coloured like phaeophytins. This is one of reasons why green vegetables (such as beans, peas and broccoli) have to be blanched to destroy the enzyme activity before preservation by freezing.

Phytol formed by hydrolysis of chlorophylls can further dehydrate to various phytadienes, an example of which is neophytadiene (**9-18**). Microbial degradation of phytol in the rumen of ruminants gives branched fatty acids (see Section 3.3.3.3.2).



9-18, 7,11,15-trimethyl-3-methylidenehexadec-1-ene

The majority of manufacturing processes and storage of green fruits and vegetable result in a greater or lesser extent of chlorophylls degradation, which is accelerated by temperature, light and ionising radiation, the presence of acids and some enzymes. The rate of chlorophyll degradation depends on the blanching or sterilisation time and temperature before freezing. Changes occurring during blanching of green beans and sterilisation of spinach are evident from the data presented in Table 9.9. Some degradation of chlorophylls occurs even during cold storage of vegetables. Deeper changes take place when the material is exposed to heat in an acidic environment, which results in the formation of phaeophytins and other degradation products. For example, the main pigments in fermented cucumbers are phaeophorbides, followed by phaeophytins, chlorophylls and chlorophylides. Phaeophorbides and phaeophytins are also the main pigments in fermented olives.

The negative effect of light is reflected especially during drying. Fruits and vegetables pre-treated with sulfur dioxide to prevent enzymatic browning reactions are much more sensitive. If the vegetables are stored in transparent containers, photooxidation continues. Ionising radiation also has a negative effect. Chlorophyll degradation is also affected by certain enzyme systems. For example, changes in chlorophyll pigments during storage of green beans and peas are attributed to degradation of chlorophyll pigments by lipoxygenase, which leads to the formation of free radicals and chlorophyll degradation.

Phaeophytinisation and other reactions described above negatively affect the sensory quality of foods containing chlorophyll pigments. The main principles of colour stabilisation are based on the positive effect of added alkaline agents (such as carbonates),

Table 9.9 Changes in chlorophylls and phaeophitins in vegetable processing (% of total pigment content in the original material).

Pigment	Green beans		Spinach	
	Raw	Blanched (100 °C/240 s)	Raw	Sterilised (127 °C/96 s)
Chlorophyll *a*	49	37	48	27
Chlorophyll *b*	25	24	20	16
Phaeophytin *a*	18	29	26	47
Phaeophytin *b*	8	10	6	10

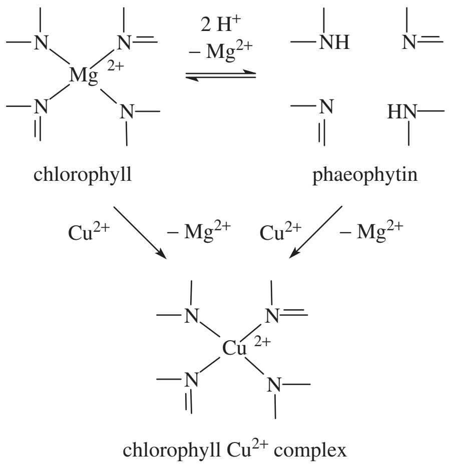


Figure 9.5 Formation of Cu^{2+} chlorophyll complex.

HTST (High Temperature Short Time) sterilisation and enzymatic conversion of chlorophylls to chlorophylides or a combination of these methods. It is also necessary to combine these procedures with storage of products at low temperatures.

Chlorophyll pigments are readily extracted with the oil from oil seeds and impart a greenish colour to the crude oil. If this oil is then processed by conventional refining techniques, chlorophylls are converted into phaeophytins, which gives the oil a dark, dull brown colour and contributes to an off-flavour. In the light, chlorophyll pigments may promote formation of oxygen radicals and speed up oil oxidation and reduce its storage stability. In oils protected from light, however, chlorophylls act as primary antioxidants. Chlorophyll pigments also act as catalyst poisons in oil hardening. Together with other undesirable substances, chlorophylls are therefore removed during refining (bleaching). Chlorophylls, however, are prominent pigments in extra virgin and virgin olive oils that are not refined. The chlorophyll content in olive oil depends on genetic factors (olive variety), geographical origin, stage of fruit ripeness (olives picked green produce greener oil), environmental conditions, the extraction process and storage conditions. A component of the unsaponifiable fraction of oils (especially of olive oils) is phytol.

For example, the main pigments of rapeseeds are chlorophyll *a* and *b*, minority pigments are phaeophytin *a*, phaeophorbide *a* and methylphaeophorbide *a*. Crude rapeseed oils contain as the main pigment phaeophytin *a*, smaller amounts of pyrophaeophytin *a* and the minority pigments are phaeophorbide *a*, methylphaeophorbide *a* and phaeophytin *b*. The total content of chlorophyll pigments in crude oils is 5–50 mg/kg. Refined (bleached) rapeseed oils may typically contain only traces (<1 mg/kg) of chlorophyll pigments. Soya oil typically contains less than 1 mg/kg of chlorophylls. The olive oil concentrations of chlorophylls vary over a wide range, from 2 to 76 mg/kg.

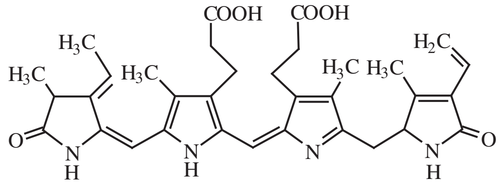
Chlorophylls form complexes with certain metals, for example, with Zn^{2+}, Cu^{2+} and Sn^{2+} (Figure 9.5), in which these metals, unlike magnesium, are bound tightly and are not released during manufacturing processes. The complexes are not cleaved even in the human digestive tract, and the metals are therefore not utilised. In earlier technological practice, the transformation of chlorophyll to phaeophytin and the related undesirable colour changes were prevented by the exchange of magnesium ion for copper ion in a solution of cupric sulfate. Today, this procedure is not used for health reasons, but the use of a chlorophyllin copper complex is permitted in some countries for food colouring. Grey–brown Sn^{2+} complexes sometimes occur in canned fruits and vegetables in containers made of tinplate. Phaeophytins, chlorophylides and phaeophorbides may also react with metal ions.

9.2.3 Phycobilins

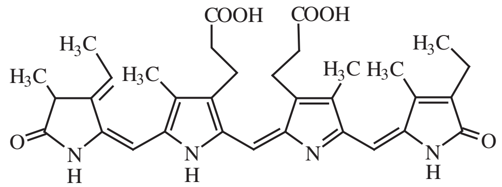
Algal bilins (phycobilins or phycobiliproteins) are fluorescent, water-soluble linear complexes of tetrapyrrole with proteins. The range of colours of phycobilins is extremely wide and depends on the source and other factors. Pigments obtained from blue–green algae (of the class Cyanophyta) were originally called C-phycobilins, and pigments of red algae (of the class Rhodophyta) were R-phycobilins. Phycobilins are divided into three main groups:

- red **phycoerythrins**
- blue **phycocyanins**
- blue **allophycocyanins**.

Phycoerythrins include four groups of compounds: C-, R- and B-phycoerythrins of red algae of the order Bangiales and cryptomonad erythrins of the algae class Cryptophyta. Also identified are C-, R- and cryptomonad phycocyanins (algae of the class Cryptophyta contain only phycoerythrins). Allophycocyanin is the only pigment. The native forms of phycobilins are composed of several bilin chromophores attached to one or two apoproteins (called α and β). Chromophores of red phycoerythrins are called phycoerythrobilins (**9-19**), chromophores of blue phycocyanins and allophycocyanins are called phycocyanobilins (**9-20**). The structure of phycobilins is very similar to the structure of mammalian bile pigments biliverdin and bilirubin, which arise as products of the catabolism of haem pigments. To date, phycobilins have not been used for food colouring in practice, but are regarded as promising pigments.



9-19, phycoerythrobilin



9-20, phycocyanobilin

9.3 Other nitrogen pigments

In addition to tetrapyrrole pigments, foods contain many other nitrogenous pigments. These pigments are theoretically derived from:

- indole
- isoquinoline
- pyrimidine.

The indole pigments include widespread animal pigments **melanins**, but also dyes of plant origin, such as **indigo**, which is not used for colouring food. Other significant indole (dihydroindole or derived dihydropyridine) pigments are water soluble red and yellow plant pigments, which are collectively called **betalains**. The only important pigment of isoquinoline type is the yellow alkaloid berberine. Substituted pyrimidines include three main groups of pigments with four nitrogen atoms in the molecule:

- purines
- pterines
- isoalloxazines.

Compounds structurally related to pterines are **phenazine** and **phenoxazine** derivatives; the most prominent representative of **isoalloxazines** is riboflavin.

9.3.1 Indoles and related compounds

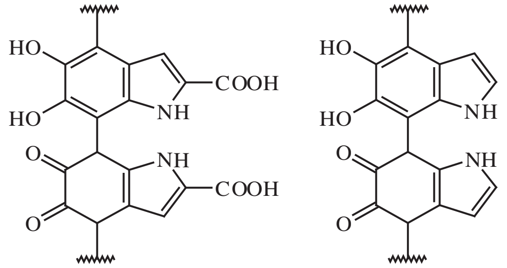
9.3.1.1 Melanins

Melanins are an important group of pigments found in all living organisms. They are divided into three basic groups (combined melanins are products of co-polymerisation of nitrogen-containing and sulfur-containing melanogens):

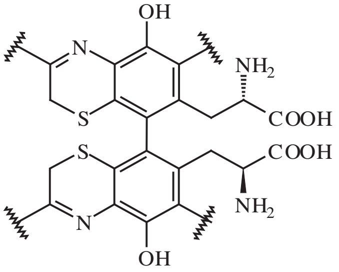
- **eumelanins** (polymerisation products of nitrogen melanogens)
- **phaeomelanins** (polymerisation products of sulfur melanogens)
- **allomelanins** (polymerisation products of polyphenols).

Melanins are formed by radical reactions *in vivo* in a process called melanogenesis, probably as a protection against harmful environmental effects upon the organism. For example, mammalian eumelanins efficiently absorb UV radiation and protect cells against reactive forms of oxygen and peroxyl radicals arising from lipids. Melanogenesis in pathogenic fungi and bacteria is associated with increased virulence, and melanins in bacteria are involved in fixation of atmospheric nitrogen.

Brown to black eumelanins (**9-21**) and yellow to red phaeomelanins (**9-22**) in various stages of oxidation and polymerisation are common pigments of skin, hair, eyes, feathers and scales of mammals, birds, reptiles, amphibians, fish and other organisms. Eumelanins and phaeomelanins in human skin arise in the basal layer of epidermis in the specialised cells melanocytes. In melanocytes, melanins occur in the cytoplasmic vacuoles, melanosomes, often in complexes with metals (Fe, Cu and Zn) and proteins. Melanins are often accompanied by other pigments, resulting in countless shades of black, brown, yellow and red colours. Phaeomelanins and eumelanins also contribute to the anomalous colour effects, such as iridiscence (interference) of colours often seen in the butterflies, reptiles and fish (they look and act like a transparent pearl, but play with the light and reflect colour with mysterious effects). Their biosynthesis, as well as biosynthesis of betalains, starts from L-tyrosine and continues via



9-21, basic structures of eumelanins



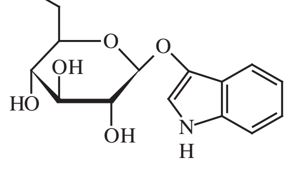
9-22, basic structure of phaeomelanins

3,4-dihydroxy-L-phenylalanine (known as L-DOPA or L-dopa) and other intermediates. Important intermediates are L-dopaquinone, 5,6-dihydroxyindole-2-carboxylic acid, its decarboxylation product 5,6-dihydroxyindole and related quinones (melanogens), which are further oxidised and polymerised to eumelanins. In the presence of thiols, such as cysteine and reduced glutathione, dopaquinone yields cysteinyl-L-dopa and its oxidation leads to benzothiazines, which polymerise to phaeomelanins.

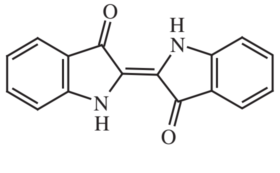
Brown to black allomelanins are a heterogeneous group of melanin pigments, which are common in many fungi, higher plants and even in some animals. They include nitrogen-free polymeric products formed from phenols and quinones that are located, for example in nut shells, leaves of plants in the final stage of evolution (senescence), and the bark of trees. Some allomelanins formed by condensation of quinones with amino acids (proteins) may also contain nitrogen and sulfur. Many plant materials form allomelanins during storage (such as bananas), tissue injury (such as apples and potatoes) or processing (e.g. during green tea fermentation) in enzymatic browning reactions. Quinones formed by oxidation of phenols and by activity of soil microorganisms are precursors of high molecular weight humic acids and related compounds. Structurally similar, but simpler brown melanins also have other functions. Such melanins are, for example, components of the dark brown pigments derived from the ink sac of some cephalopods, such as the common cuttlefish (*Sepia officinalis*) and squids (of the order Teuthida) that can be found in special sauces consumed in the Mediterranean region.

9.3.1.2 Indigoid pigments

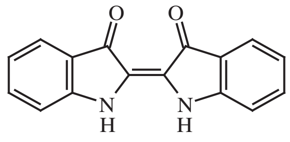
Indigo, also known as *trans*- or (*E*)-indigo or 2,2′-bis(2,3-dihydro-3-oxoindolylidene), has been valued since ancient times as a natural blue textile dye. It occurs in various types of indigo trees (such as true indigo *Indigofera tinctoria*, Fabaceae) naturalised to tropical and temperate Asia and Africa. Indigo may be also obtained from European woad (*Isatis tinctoria*), a plant native to south-eastern Europe and western Asia. True indigo trees contain the colourless indigo precursor indoxyl-β-D-glucopyranoside called indican (**9-23**). In addition to (*E*)-indigo (**9-24**), some other structurally related minor compounds include blue *cis*- or (*Z*)-indigo (**9-25**), red indirubin (**9-26**) and isoindirubin (**9-27**) and brown isoindigo (**9-28**). Fermentation (hydrolysis by saccharases) yields an unstable 3-hydroxyindol (indoxyl), which oxidises in air and provides indigo. The main precursor of indigo in woad is isatan B (isatan-β-D-gluconic acid), while indican is a minor component.



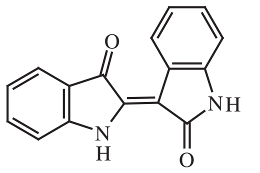
9-23, indican



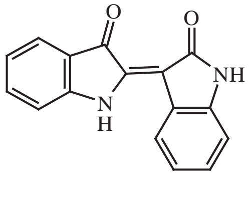
9-24, (*E*)-indigo



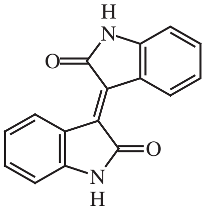
9-25, (*Z*)-indigo



9-26, indirubin



9-27, isoindirubin



9-28, isoindigo

The starting compound for the biosynthesis of indican is probably tryptophan, from which 1-(2-carboxyphenylamino)-1′-deoxy-D-ribulose 5′-phosphate, indole and 3-hydroxyindole are formed.

Sulfonation of natural indigo produces the dark blue, water soluble pigment known as indigo carmine. Indigo carmine is used in some countries as a food colorant, but its use in the EU is not permitted. Indigo carmine is possibly a weak local sensitiser (slightly irritating to the eyes) and is harmful to the respiratory tract if inhaled (occupational asthma has been reported).

9.3.1.3 Betalains

Betalains are a group of about 100 water-soluble purple, red, orange and yellow pigments of higher plants and some higher fungi. Two groups of betalains are differentiated:

- **betacyanins** (formerly called nitrogen anthocyanins) resembling anthocyanins with their purple and red colour
- yellow and orange **betaxanthins**.

9.3.1.3.1 Structure and nomenclature

All betalains have the same basic structure. The chromophore system of conjugated double bonds is derived from dihydropyridine (Figure 9.6). The individual compounds differ from each other by the structure of substituents R and R^1, which are aliphatic or part of the nitrogen heterocycles.

Betacyanins

All known betacyanins (about 50 compounds) are found exclusively as glycosides and acylated glycosides. The main aglycone of the important betacyanins is betanidin (**9-29**). In small quantities, betanidin is accompanied by its C-15 epimer isobetanidin (**9-30**), while neobetanidin (14,15-dehydrobetanidin, **9-31**) and

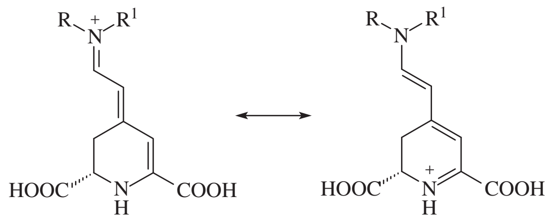
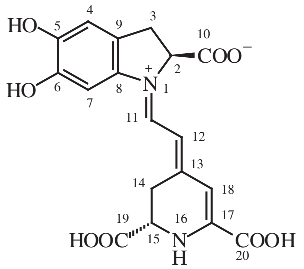
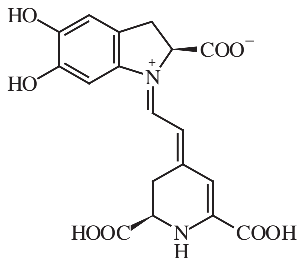


Figure 9.6 Basic structure of betalains.

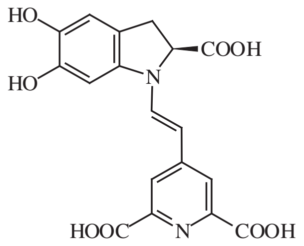
2-decarboxybetanidin (**9-32**) occur sporadically. Recent publications demonstrated the possibility of the generation of neoderivatives and 2-decarboxylated derivatives during heating of red beet and purple pitaya. Various other decarboxylated pigments may also be produced.



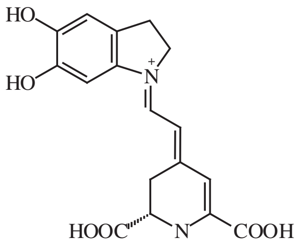
9-29, betanidin



9-30, isobetanidin



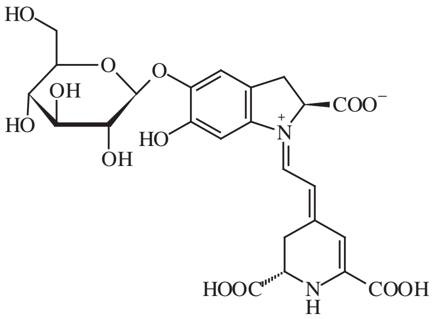
9-31, neobetanidin



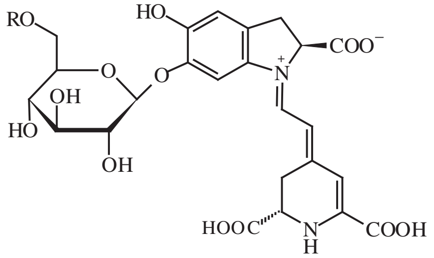
9-32, 2-decarboxybetanidin

The dominating betacyanin is 5-*O*-β-D-glucoside of betanidin, (15*S*)-betanidin-5-*O*-β-D-glucopyranoside, which is called betanin (**9-33**). It is usually accompanied by isobetanin, which is the (15*R*)-isomer of betanin. Other minor pigments are 6-sulfates of betanin and isobetanin that are known as prebetanin and isoprebetanin, respectively. A less common pigment is 6-*O*-β-D-glucoside of betanidin, gomphrenin I (**9-34**).

Less common sugars bound in betacyanins include L-rhamnose, D-glucuronic acid and β-sophorose. Amaranthin is an example of a pigment where bound at position C-5 of betanidin is the disaccharide β-D-Glc*p*A-(1→2)-β-D-Glc*p*, which is related to sophorose, β-D-Glc*p*-(1→2)-β-D-Glc*p*. The sugar component of betalains can be esterified with malonic, ferulic, *p*-coumaric, sinapic, caffeic, citric and 3-hydroxy-3-methylglutaric acids. For example, phyllocactin is betanin, which has glucose at position C-6 esterified with malonic acid, hylocerenin is esterified at position C-6 with 3-hydroxy-3-methylglutaric acid and gomphrenin II is gomphrenin I esterified with *p*-coumaric acid at position C-6 of glucose (**9-34**).



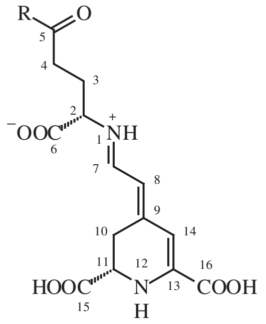
9-33, betanin, R = H
prebetanin, R = SO_3H



9-34, gomphrenin I, R = H
phyllocactin, R = $COCH_2COOH$
hylocerenin, R = $COCH_2CH(OH)(CH_3)CH_2COOH$

Betaxanthins

Betaxanthins are dihydropyridine derivatives (Figure 9.6) that arise as condensation products of betalamic acid (Figure 9.7) with amino acids or biogenic amines. Examples of such, mainly yellow, pigments are vulgaxanthin I, which is (*S*)-glutamine-betaxanthin (R = H and R^1 = glutamine residue), vulgaxanthin II (R = H and R^1 = glutamic acid residue, **9-35**), vulgaxanthin III (R = H, R^1 = asparagine residue) and vulgaxanthin IV (R = H, R^1 = leucine residue). The R^1 substituents of other pigments are derived from methionine sulfoxide (miraxanthin I), aspartic acid (miraxanthin II), tyramine (miraxanthin III), tyrosine (portulaxanthin II) or dopa (dopaxanthin). Less widespread is indicaxanthin, which is



9-35, vulgaxanthin I, R = NH_2
vulgaxanthin II, R = OH

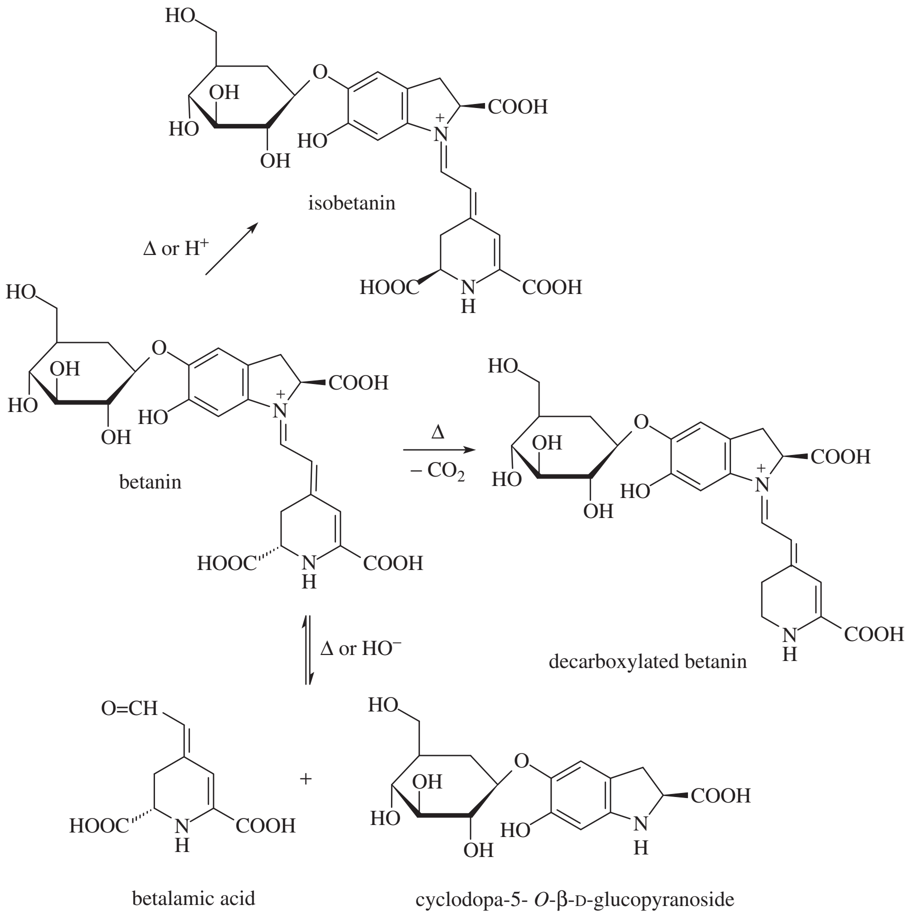
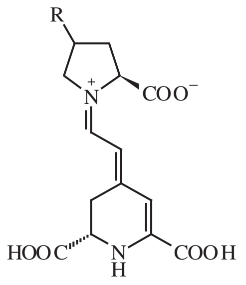
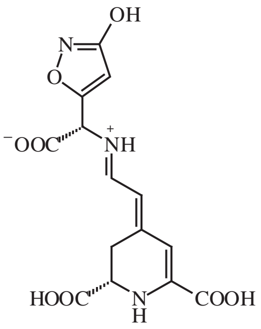


Figure 9.7 Mechanism of betanin degradation.

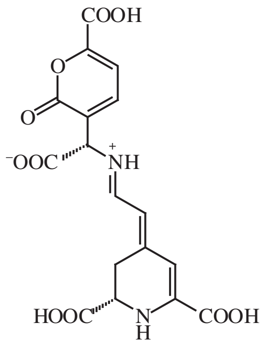
(*S*)-proline-betaxanthin (**9-36**), portulaxanthin I (**9-36**) containing 4-hydroxyproline residue and muscaurin (muscaaurin) I (**9-37**) and muscaurin II (**9-38**), which are derived from ibotenic and stizolobic acids, respectively.



9-36, indicaxanthin, R = H
portulaxanthin I, R = OH



9-37, muscaurin I



9-38, muscaurin II

9.3.1.3.2 Occurrence

Betalains occur in nature in a relatively small number of plant genera belonging to the order of flowering plants Caryophyllales. The only exceptions are plants of the pink (carnation) family (Caryophyllaceae) and of the family Molluginaceae that accumulate anthocyanins instead of betacyanins. These two types of plant pigments never occur together in one plant. The most important sources of betacyanins are the cultivated red varieties of beetroot (cultivars of *Beta vulgaris*, Amaranthaceae, formerly Chenopodiaceae), according to which these pigments were named, and a shrub with red berries known as American pokeweed (*Phytolacca americana*) of the family *Phytolaccaceae*, which is native to eastern North America.

Beetroot has an average betalains content of 0.1%. Some varieties, however, contain up to twice as much pigment, which is

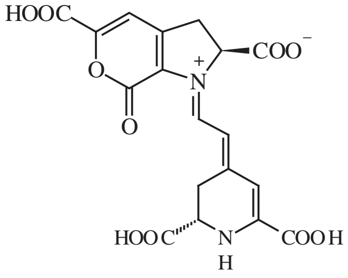
about 2 g/kg (in fresh matter). Betanin represents about 75–95% of all betacyanins of beetroot and dominates significantly over yellow betaxanthins. The main yellow pigment is vulgaxanthin I, which represents about 95% of yellow pigments, the rest are vulgaxanthin II and betalamic acid, a key intermediate of the biosynthesis and degradation of betalains (Figure 9.7). The pigment of American pokeweed was originally called phytolaccanin, but it was later identified as betanin.

The main pigment of red flowers, leaves and stems of the ornamental plant *Amaranthus tricolor* and of other species of the family Amaranthaceae, some of which are consumed for their seeds, leaves and stems, is amaranthin derived from betanidin, which is accompanied by isoamaranthin derived from isobetanidin. The main red pigments of seeds of Indian (Ceylon) spinach (*Basella alba*, Basellaceae), whose leaves are eaten as a vegetable, are gomphrenin I and gomphrenin II. Betalains also occur in edible fruits of some cacti (nopales) of the genus *Opuntia* (Cactaceae). Fruits of *O. ficus-indica*, referred to as prickly pear or Indian fig, contain yellow indicaxanthin (yellow fruits) and red betanin (red fruits). Betanin together with acylated betacyanins hylocerenin and phyllocactin occurs in fruits of pitaya (*Hylocereus polyrhizus*), also known as dragon fruit, and other domesticated species of the genus *Hylocereus* (such as *H. costaricensis* with red pulpa). Phyllocactin also occurs in flowers of several cacti, such as *Epiphyllum hybridum* (syn. *Phyllocactus hybridus*).

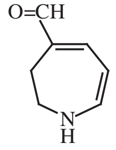
Betalains are also pigments of flowers in some plants. Flowers of the American plant four o'clock flower (*Mirabilis jalapa*, Nyctaginaceae) contain miraxanthins. 6-*O*-β-Sophorosides and other glycosides of betanidin and isobetanidin are pigments of paper-like bracts that surround flowers of the Brazilian paper plant *Bougainvillea glabra* from the same plant family. The pigment of flowers of moss-rose purslane (*Portulaca grandiflora*, Portulacaceae) native to South America is portulaxanthin.

Betalains are also pigments of some mushrooms. For example, the common mushroom (*Agaricus bisporus*, Agaricaceae) supposedly contains small quantities of the same pigments as beetroot. Following tissue damage, the light pink colour of the fungus changes to grey–black since L-dopa is oxidised to melanin pigments.

The striking orange–red pigment of the cap of fly agaric *Amanita muscaria* (Amanitaceae) is a mixture of the purple betacyanin muscapurpurin (**9-39**), orange betaxanthins muscaurins I–VII and yellow muscaflavin (**9-40**). Muscaurin I (**9-37**) and muscaurin II (**9-38**) are derived from unusual mushroom non-protein amino acids ibotenic and stizolobic acids, respectively, which are the major agaric pigments. Pigments termed muscaurins III–VII were later identified as mixtures of pigments derived from common protenogenous amino acids. Muscaurin III is a mixture of vulgaxanthin I (**9-35**), known as (*S*)-glutamine-betaxanthin, miraxanthin III, known as (*S*)-aspartic acid-betaxanthin and betaxanthin derived from 2-aminoadipic acid. Muscaurin IV is a mixture of vulgaxanthin I (**9-35**) and miraxanthin III. Muscaurin V is a mixture of vulgaxanthin II (**9-38**), known as (*S*)-glutamic acid-betaxanthin, indicaxanthine (**9-36**), known as (*S*)-proline-betaxanthin, and betaxanthins derived from valine and leucine (vulgaxanthin IV). Muscaurin VI is a mixture of vulgaxanthin II (**9-38**) and indicaxanthin (**9-36**). One of the dominant pigments, muscaurin VII, is derived from histidine.

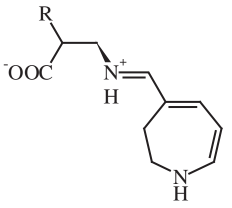


9-39, muscapurpurin



9-40, muscaflavin

Fly agaric and mushrooms of the genus Hygrocybe (*Hygrophoraceae*), such as *H. conica*, commonly known as the witch's hat, synthesise a yellow isomer of betalamic acid called muscaflavin (**9-40**), which is derived from dihydroazepine, and store it in the cup skin. In the same way that betalamic acid is involved in the formation of various betalains, muscaflavin can spontaneously condense with amino acids, with the formation of aldimine bonds, yielding yellow hygroaurins (**9-41**).



9-41, hygroaurins (R = amino acid residue)

9.3.1.3.3 Use

Only pigments from beetroot have found significant practical use, and are commercially called betanin or beetroot red. Owing to its low stability, the beetroot red is used for colouring foods with a shorter shelf-life, such as dairy and meat products (e.g. sausages from poultry meat that has a light colour), acidic beverages, such as soft drinks and some sweets. The pigment is supplied as a concentrated syrup or powder. The juice of the American pokeweed was used in the past to colour wine, which is considered falsification and is illegal.

9.3.1.3.4 Biochemistry, physiology and nutrition

The precursor of betacyanins is amino acid L-tyrosine, and correspondingly, 3,4-dihydroxy-L-phenylalanine (L-dopa) resulting from its oxidation. By a sequence of oxidation reactions via 4,5-seco-L-dopa, betalamic acid (Figure 9.7) and cyclo-3-(3,4-dihydroxyphenyl)-L-alanine (L-cyclodopa) are produced. Condensation of betalamic acid with cyclodopa yields betacyanins, while its condensation with amino acids produces betaxanthins. Muscaflavin in fungi also arises from dopa, but through the 2,3-seco-L-dopa spontaneous cyclisation. Reaction of muscaflavin with amino acids yields hygroaurins (Figure 9.8).

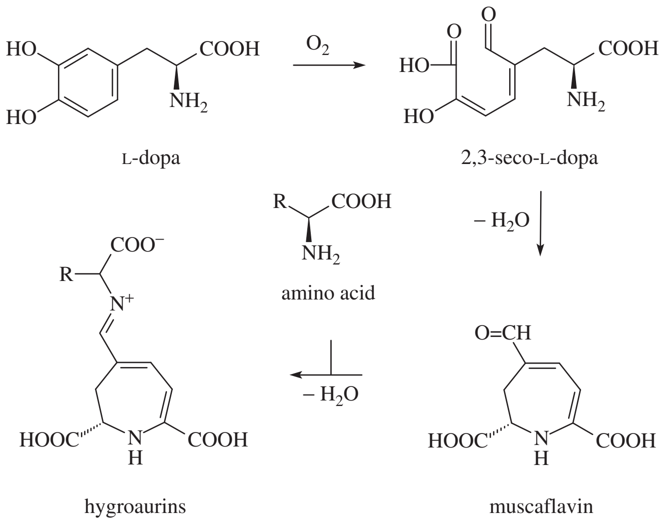
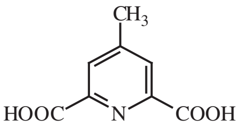


Figure 9.8 Biosynthesis of muscaflavin and hygroaurins.

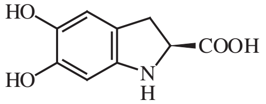
Betalains exhibit antioxidant activity and also react with free radicals. Their adverse effects were not detected; therefore beetroot pigments are generally accepted as safe food colorants. Furthermore, the maximum amount of added beetroot red is far less than the amount normally consumed by eating beetroot.

9.3.1.3.5 Properties and reactions

Beetroot betacyanins have a very intense colour, but are sensitive to oxidation (on light and especially in the presence of divalent and trivalent metals) and the presence of sulfur dioxide (that decolourises betacyanins). In the absence of these agents, the rate and extent of betacyanin degradation depends mainly on water activity and pH (Figure 9.7). They exhibit the highest stability in solutions of pH 4–5, but at pH > 7 are rapidly degraded. In acidic solutions, betacyanins hydrolyse to the corresponding aglycones and glucose. The degradation products in alkaline solutions are 4-methylpyridine-2,6-dicarboxylic acid (**9-42**), formic acid and (*S*)-5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid (1*H*-indole-2-carboxylic acid, **9-43**). The latter compound also arises, together with the corresponding amino acids or amines, as a degradation product of betaxanthins in aqueous solutions.



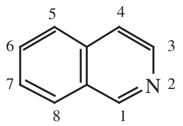
9-42, 4-methylpyridin-2,6-dicarboxylic acid



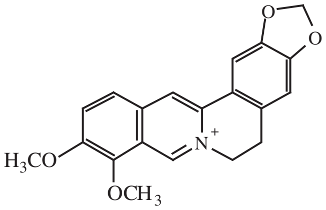
9-43, (*S*)-5,6-dihydroxy-2,3-dihydroindole-2-carboxylic acid

9.3.2 Isoquinolines

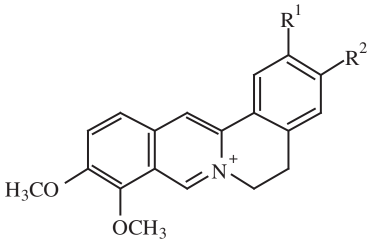
The isoquinoline (**9-44**) or benzyltetrahydroisoquinoline skeleton, respectively, can be found in the tetracyclic alkaloid berberine, also called umbellatin (**9-45**). Together with related alkaloids, berberine occurs in all parts of the European barberry shrub (*Berberis vulgaris*, Berberidaceae) as the main component, especially in the cortex, but also in leaves and immature fruits. Barberry alkaloids are carriers of the characteristic yellow colour of barberry wood and bark, but do not practically influence the colour of edible berries. The mildly poisonous cortex of the roots, containing 12–15% of alkaloids, was used in the past as a drug for medical purposes (the bark of stem contains 5.5–8% and the bark of branches about 3.5% of the mixture of alkaloids). The main components of the root bark are quaternary bases berberine (**9-45**), jatrorrhizine, columbamine, palmatine (**9-46**) and tertiary bases oxyacanthine (**9-47**) and berbamine (**9-48**).



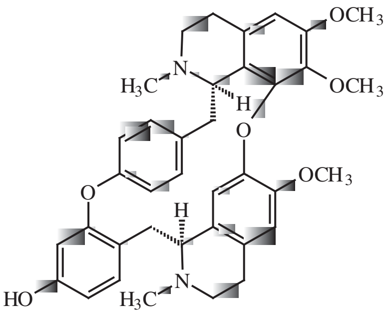
9-44, isoquinoline



9-45, berberine

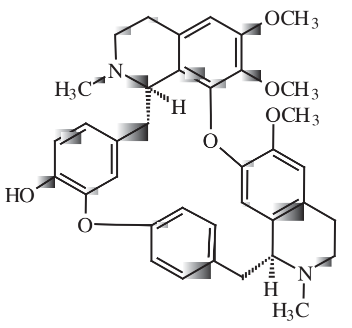


9-46, jatrorrhizine, $R^1 = OCH_3$, $R^2 = OH$
columbamine, $R^1 = OH$, $R^2 = OCH_3$
palmatine, $R^1 = OCH_3$, $R^2 = OCH_3$



9-47, oxyacanthine

Berberine also occurs in a widespread evergreen shrub called Oregon-grape (*Mahonia aquifolium*, Berberidaceae) related to the barberry and in goldenseal, also known as orangeroot (*Hydrastis canadensis*, Ranunculaceae).



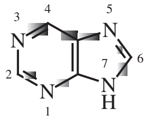
9-48, berbamine

Biosynthesis of berberine and related alkaloids is based on the transformation of phenylalanine. The tetracyclic skeleton (derived from benzytetrahydroisoquinoline) arises by incorporation of the *N*-methyl group (so-called berberine bridge) in alkaloid reticulin, which is the immediate precursor of berberine.

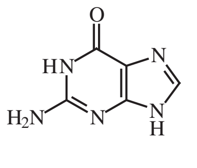
Various Asian species of barberry, such as *B. amurensis* and *B. asiatica*, are used in medical preparations in India and the Far East. Berberine reportedly promotes the excretion of urine and the relief of smooth muscles (spasmolytic effect) in renal diseases and urinary tract infections, increases production and secretion of bile (choleretic effect). Also important are its sedative effect and activity during violent diarrhoea. Berberine also acts against infections caused by amoebas and parasitic trypanosomes (causing sleeping sickness), and has been used in the treatment of psoriasis. Overdose or overuse leads to transient irritation of the central nervous system, stupor and diarrhoea and can cause kidney damage. Because of its relative toxicity and side effects, the use of drugs containing berberine is restricted in many countries. The use of berberine as a yellow food pigment and bittering agent for liqueurs and spirits was reported by a Japanese company, Kakko Honsha, in 1980. In the EU, the berberine content in aromatised foods is restricted by law. The tolerated content (maximum level), originating exclusively from natural sources, is for alcoholic beverages 10 mg/l, and 0.1 mg/l (0.1 mg/kg) for other foods and beverages.

9.3.3 Purines

Pigments derived from purine (**9-49**), for example guanine (**9-50**), xanthine (**9-51**) and uric acid (**9-52**), are very important pigments of the animal kingdom. The actual compounds are colourless, but in the form of granules or microcrystals are the bases of white, cream and silvery semi-transparent pigments that are found, for example, in fish scales.

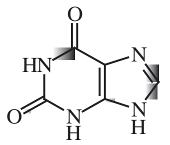


9-49, purine

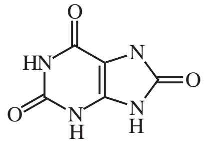


9-50, guanine

Biosynthesis of purines proceeds by a multi-step mechanism via inosine 5′-monophosphate (IMP). Free purines are formed by



9-51, xanthine

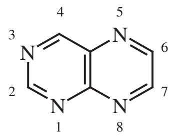


9-52, uric acid

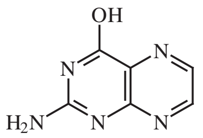
hydrolysis of the corresponding nucleotides; uric acid is a product of their catabolism.

9.3.4 Pterines

The basic skeleton of pterine pigments is pteridine (**9-53**). Pteridine substituted in position C-2 with an amino group and in position C-4 with a hydroxyl group (tautomeric oxo group) is called pterine (**9-54**). Many biologically active forms of pteroylglutamic (folic) acid are natural pterines, and have the function of vitamins, but due to the quantities in which they occur in foods they have no importance as yellow food pigments.

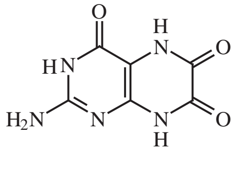


9-53, pteridine

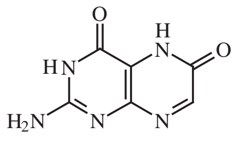


9-54, pterine

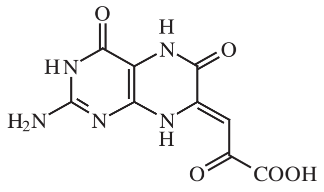
Important coloured pterines include white leucopterine (**9-55**), yellow xanthopterine (**9-56**) and red-orange erythropterine (**9-57**), which are pigments of butterfly wings. Leucopterine is found in wings of white butterflies of the genus *Pieris* (such as small white), xanthopterin in yellow butterflies of the genus *Gonepteryx* (brimstones) and erythropterin is a pigment of butterflies of the family *Nimphalidae* (such as Red Admiral, *Vanessa atalanta*) and some insects (e.g. wasps, *Vespula* spp.). Yellow, orange and red pterine pigments also occur in some shellfish, fish, amphibians and reptiles. Pteridines also act as shielding pigments of eyes in some invertebrates.



9-55, leucopterine



9-56, xanthopterine

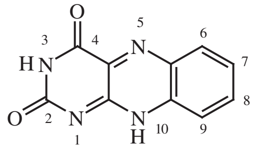


9-57, erythropterine

Biosynthesis of pteridines is based on guanosine 5′-triphosphate transformation. Compounds belonging to folacins arise analogously.

9.3.5 Isoalloxazines

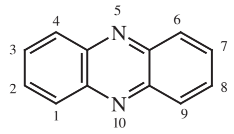
Numerous flavins (and flavoproteins) that are derived from isoalloxazine (**9-58**) are also biologically significant pigments. The most important representative of this group of pigments is riboflavin (vitamin B_2), which is also used as a yellow food pigment.



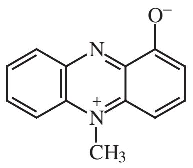
9-58, isoalloxazine

9.3.6 Phenazines

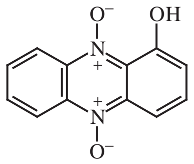
Pigments derived from phenazine (**9-59**) are related to flavin and isoalloxazine pigments. They are synthesised by some bacteria, mainly by bacteria belonging to the genera *Pseudomonas* and *Streptomyces*. With the exception of dark blue pyocyanin (**9-60**) and blue–violet iodinin (**9-61**), most phenazines are yellow pigments. Occasionally, these pigments may be found in animal tissues in relation to the invasion of microorganisms (e.g. in infected wounds).



9-59, phenazine



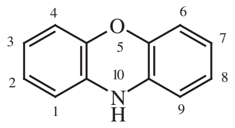
9-60, pyocyanin



9-61, iodinin

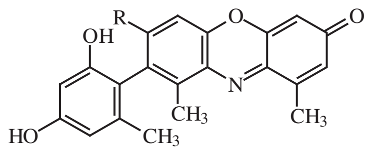
9.3.7 Phenoxazines

Phenoxazine (**9-62**) pigments are compounds structurally similar to phenazines. Phenoxazines occur mainly in lichens (symbiotic organisms of fungi belonging to the phylum Ascomycota, algae or cyanobacteria), mostly in the form of uncoloured precursors (different depsides). To a lesser extent, phenoxazines occur in higher fungi of the Polyporaceae family. Pigments extracted from lichens with urine (a source of ammonia) or ammonia solutions and oxidised by air oxygen have been used as textile dyes from time immemorial. Another pigment of the same type acquired from the same lichen species, such as *Roccella tinctoria* (Roccellaceae) is litmus (a mixture of several related compounds), one of the oldest pH indicators, which was once used to colour various drinks.

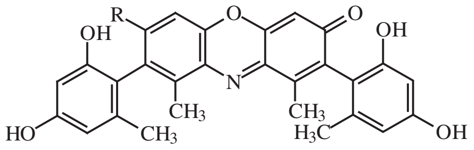


9-62, phenoxazine

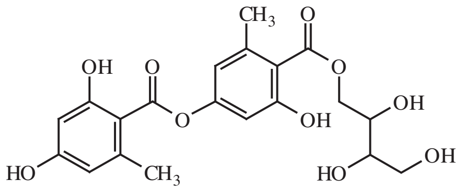
An important group of phenoxazine pigments are orcein pigments known as orcein or orchil, which occur in many parts of the world, but come mainly from the Azores and the Canary Islands. Orcein is a purple-to-red pigment extracted from lichens (orchella weeds) of the genera *Roccella* and *Orchella*. It is a mixture of different hydroxyphenoxazones and aminophenoxazones. Examples of these components are α-hydroxy and α-amino orceins (**9-63**) and β-hydroxy and β-amino orceins (**9-64**). Their precursors in lichens are some aromatic acid depsides, such as lecanoric acid (**9-65**) and erythrin (**9-66**) that produce as a degradation product orcinol (**9-67**) and analogous products, which on condensation produce orcein pigments. The dye is very slightly soluble in water, but soluble in ethanol. Orcein (orchil) can be used to dye wool and silk, and is also approved as a food colorant (with E number E121), but is banned throughout the EU. Sulfonation of orcein produces a water-soluble pigment, which is used for colouring soft drinks and confectionery.



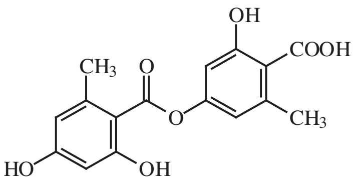
9-63, α-hydroxy orcein (R = OH)
α-amino orcein (R = NH_2)



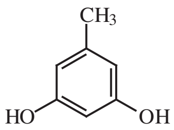
9-64, β-hydroxy orcein (R = OH)
β-amino orcein (R = NH_2)



9-65, lecanoric acid

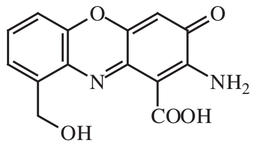


9-66, erythrin



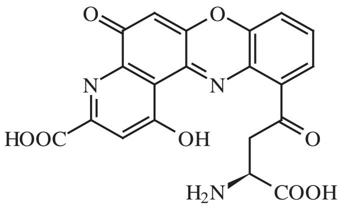
9-67, orcinol

An example of a phenoxazine pigment occurring in higher fungi is the cinnabar red pigment of the wood-rotting fungus *Pycnoporus cinnabarinus*, 2-amino-3*H*-phenoxazin-3-one-1,9-dicarboxylic acid named cinnabaric acid (**9-68**).



9-68, cinnabaric acid

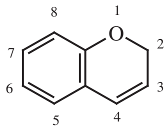
Phenoxazines also include yellow-to-brown and brownish-red pigments found in invertebrate animals and some vertebrates, for example as shielding pigments of eyes. The most important pigments are **ommochromes** that arise from tryptophan via kynurenine and 3-hydroxykynurenine. An example is a yellow pigment xanthommatin (**9-69**) containing bound aspartic acid.



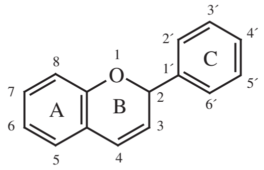
9-69, xanthommatin

9.4 Flavonoids

Flavonoids or flavonoid substances are a very large group of plant phenols containing two benzene rings (ring A and C) in the molecule, associated by a three-carbon chain. The number of flavonoid compounds is now estimated at 5000 and new compounds are still being found in various plant sources. Most flavonoid compounds have the C_3 chain as a part of a heterocyclic ring derived from 2*H*-pyran (ring C). These flavonoids are therefore derived from the oxygen-containing heterocyclic compounds 2*H*-chromene (**9-70**), substituted in position C-2 by phenyl group, which is called flavan (**9-71**) having an arrangement of C_6–C_3–C_6. Compounds of this type are often called 1,3-diarylpropanoids. Typically, all three rings of flavonoids are substituted with hydroxyl or methoxyl groups, and individual derivatives differ only in the degree of substitution and oxidation. Flavonoids occur as free compounds, but more frequently as glycosides, acylated glycosides, and polymers. The properties of flavonoids are very different from those of other phenolic pigments; therefore flavonoids are described as a separate group of plant pigments. Other important plant phenols are included in the sections dealing with quinoid pigments, natural antioxidants, natural toxicants and various flavour-active substances.



9-70, 2*H*-chromene



9-71, flavan

Depending on the degree of oxidation and C-3 chain substitution the following basic structures of flavonoids can be identified:

- **catechins** (flavan-3-ols)
- **leucoanthocyanidins** (flavan-3,4-diols)
- **flavanons**
- **flavanonols**
- **flavons**
- **flavonols** (dihydroflavones)
- **anthocyanidins**.

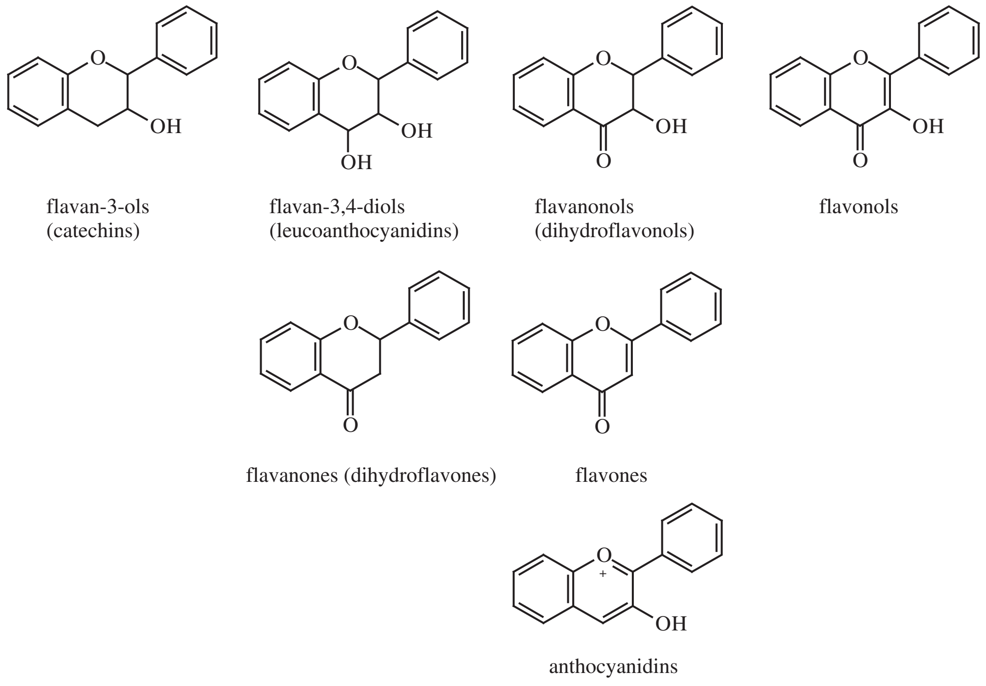
The basic structure of these flavonoid compounds is shown in formulae **9-72**. Their degree of oxidation increases along the row from left to right, along with the colour intensity. The compounds listed in the columns below have the same degree of oxidation. A special case are anthocyanidins that contain a system of conjugated double bonds, therefore they are flavylium or 2-phenylbenzopyrylium (2-phenylchromenylium) cations.

In several cases, the six-membered heterocyclic ring B exists in the isomeric open form, or can be substituted by a five-membered heterocyclic ring. The structurally related compounds, in which an aliphatic C-3 chain or a chain, which is partially included into the furan ring, connects the rings A and C are divided into:

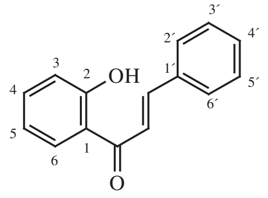
- **chalcones** and **dihydrochalcones**
- **aurones**.

Structures of these flavonoids are given in formulae **9-73**, **9-74** and **9-75**.

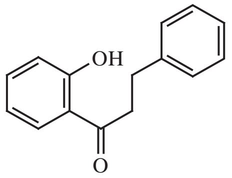
Less common compounds with ring B associated with pyran ring C in position C-3 (derived from isoflavan) are called **isoflavonoids** (also known as 3-phenylchromen-4-ones or 1,2-diarylpropanes, **9-76**). If the connection between these two rings is in position C-4, the corresponding compounds, derived from neoflavan, are called **neoflavonoids** or 4-phenylcoumarins, 5,6-benzo-2-pyrones or 1,1-diarylpropanes (**9-77**).



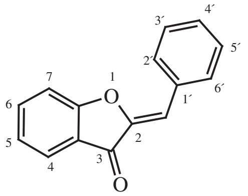
9-72, common structures of main flavonoid compounds



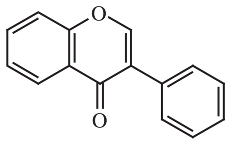
9-73, chalcones



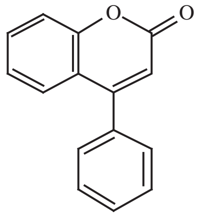
9-74, dihydrochalcones



9-75, aurones



9-76, isoflavonoids



9-77, neoflavonoids

Only certain flavonoids are important as natural plant pigments; others act as taste-active compounds, as they are precursors of astringent and bitter substances or have various important biological effects. All coloured flavonoids were previously logically divided into two large groups according to their colour, the red and blue **anthocyanins** and yellow **anthoxanthins**. Their names were derived from the Greek word flower (anthos), blue (cyaneos) and yellow (xanthos). Names of flavones and other yellow flavonoids are derived from the Latin word flavus (yellow). Chalcones and aurones were previously called anthochlor pigments or **anthochlors** (the Greek word chloros means green).

Catechins and leucoanthocyanidins are colourless compounds, but the brown pigments that arise from these flavonoids in enzymatic browning reactions are important pigments of many foods. Colourless leucoanthocyanidins also give rise to the corresponding coloured anthocyanidins during processing of fruits and vegetables in acidic solutions. Oligomers of catechins and leucoanthocyanidins with bitter taste are classified as condensed tannins. Flavanones and flavanonols are colourless or pale yellow compounds, and do not have great significance as plant pigments. Some flavanones, however, are important bitter components of grapefruits. The most important flavonoid pigments are yellow flavones and flavonols and anthocyanins in particular that are mostly red (also yellow or orange), purple and blue anthocyanins. Chalcones and dihydrochalcones are yellow pigments, aurones are golden yellow pigments mostly of plant flowers, but as food pigments they are not particularly important. Other pale yellow pigments, isoflavones, similarly have negligible importance as pigments, but they show important biological properties (oestrogenic activity, see Section 10.4). They are found only in certain commodities (notably soya beans and derived products).

9.4.1 Anthocyanins

Anthocyanins are the most widespread and numerically a very large group of hydrophilic plant pigments. So far about 300 different anthocyanins have been identified in nature. Many fruits, vegetables, flowers and other plant materials owe their attractive orange, red, purple and blue colour, which increases their consumer popularity, to this group of water-soluble pigments.

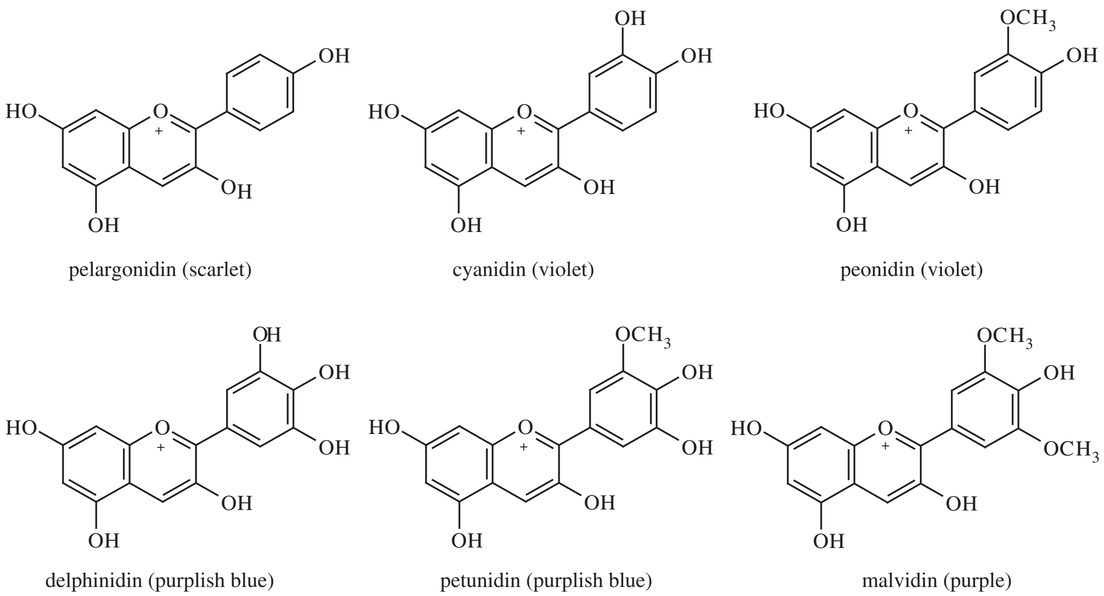
9.4.1.1 Structure and nomenclature

Anthocyanins are glycosides of different aglycones that are called anthocyanidins. All anthocyanidins are derived from one basic structure, which is flavylium (2-phenylbenzopyrylium or 2-phenylchromenylium) cation (**9-72**). It is reported that 17 different anthocyanidins exist in nature. All compounds are substituted in position C-4 by a hydroxyl group and they mutually differ by substitutions at positions C-3, C-5, C-6, C-7, C-3′ and C-5′ and in positions C-5, C-7, C-3′and C-5′ methoxyl groups may occur. Table 9.10 lists the 15 most common anthocyanidins, the last two compounds are 6-hydroxyanthocyanidin (abbreviated 6-OHCy) and 5-methylcyanidin (5-MCy). The most important compounds occurring in foods are six anthocyanidins (**9-78**) with a hydroxyl

Table 9.10 Overview of anthocyanidin structures.

Name[a]	Abbreviation	Substituents					
		C-3	C-5	C-6	C-7	C-3′	C-5′
Apigeninidin	Ap	H	OH	H	OH	H	H
Luteolinidin	Lt	H	OH	H	OH	OH	H
Tricitinidin	Tr	H	OH	H	OH	OH	OH
Pelargonidin	Pg	OH	OH	H	OH	H	H
Aurantinidin	Au	OH	OH	OH	OH	H	H
Cyanidin	Cy	OH	OH	H	OH	OH	H
Peonidin	Pn	OH	OH	H	OH	OCH_3	H
Rosinidin	Rs	OH	OH	H	OCH_3	OCH_3	H
Delfinidin	Dp	OH	OH	H	OH	OH	OH
Petunidin	Pt	OH	OH	H	OH	OCH_3	OH
Pulchellidin	Pl	OH	OCH_3	H	OH	OH	OH
Europinidin	Eu	OH	OCH_3	H	OH	OCH_3	OH
Malvidin	Mv	OH	OH	H	OH	OCH_3	OCH_3
Hirsutidin	Hs	OH	OH	H	OCH_3	OCH_3	OCH_3
Capensinidin	Cp	OH	OCH_3	H	OH	OCH_3	OCH_3

[a] Most important anthocyanidins are indicated in bold.



9-78, common anthocyanidins

group at position C-3. In descending frequency of occurrence these anthocyanidins are: cyanidin (named after the Latin name for the cornflower (*Centaurea cyanus*, Asteraceae), pelargonidin (found in geraniums, *Pelargonium* spp., Geraniaceae), peonidin (found in peonies, *Paeonia* spp., Paeoniaceae), delphinidin (occurring in delphiniums, *Delphinium* spp., Ranunculaceae), petunidin (found in petunias, *Petunia* spp., Solanaceae) and malvidin (found in mallow, *Malva* spp., Malvaceae), formerly also known as oenidin.

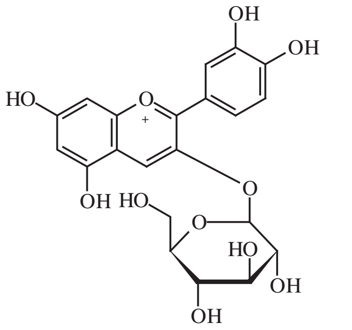
Materials that contain anthocyanins generally have the colour of their aglycones. Free aglycones of anthocyanidins occur in plant tissues rarely and only in trace amounts as anthocyanin hydrolysis products and products derived from leucoanthocyanidins. In all plant materials, the main pigments are glycosides and acylated glycosides of anthocyanins. Present in some fruits and vegetables are anthocyanins derived from a single anthocyanidine (e.g. in apples, red cabbage varieties and elderberries these are cyanidin glycosides), but sometimes pigments are derived from several different anthocyanidins. For example, blackcurrant anthocyanins are delphinidin and cyanidin derivatives, while strawberry anthocyanins are derived from pelargonidin and cyanidin.

The anthocyanin pigments contain five monosaccharides that include, in descending order of frequency, D-glucose, L-rhamnose, D-galactose, D-xylose and L-arabinose. Sugars are always bound in position C-3, and if another hydroxyl group is glycosylated, it is the hydroxyl at C-5 (the bound sugar is glucose and less often rhamnose). Free hydroxyl at C-3 destabilises the anthocyanidin chromophore, so the hydrolysis of sugar bound as *O*-glycoside at C-3 results in rapid and irreversible decomposition of anthocyanidin, and leads to discoloration. In exceptional cases, sugars are also bound in positions C-7, C-3′, C-5′ and C-4′ as mono- (mainly glucose), di- or trisaccharides. The most common disaccharides are rutinose, sambubiose, sophorose, laminaribiose and gentiobiose.

Depending on the number of bound sugar molecules, anthocyanins are divided into 18 groups, of which the most important are:

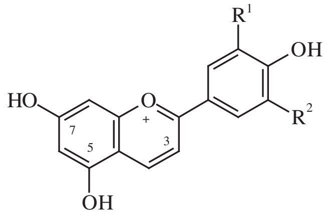
- monosides with glucose, galactose, rhamnose or arabinose in position C-3 (3-monosides);
- biosides with disaccharides (such as rutinose, sambubiose, sophorose, gentiobiose, neohesperidose, laminaribiose, robinobiose and other disaccharides) bound in position C-3 (3-biosides);
- triosides with linear or branched trisaccharides bound in position C-3 (3-triosides);
- 3,5-diglycosides with monosaccharides at positions C-3 and C-5;
- 3,7-diglycosides with monosaccharides at positions C-3 and C-7;
- 3-biosides-5-monosides with disaccharide in position C-3 and monosaccharide in position C-5.

The most frequently occurring anthocyanine pigments are cyanidin-3-*O*-glycosides, which occur approximately three times more frequently than 3,5-di-*O*-glycosides. The type of sugar has little effect on the chemical properties of pigments, but much more significant is the position in which the sugar is bound. An example of 3-*O*-glycosides is cyanidin-3-*O*-β-D-glucopyranoside (**9-79**).



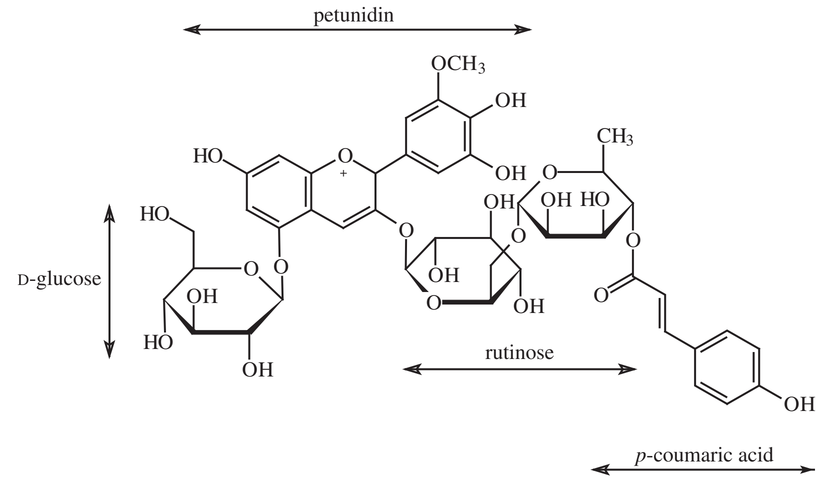
9-79, cyanidin-3-*O*-β-D-glucopyranoside

Besides the common anthocyanins, some unusual compounds differing from the normal structures in the substituent position are also found in nature. Examples of rare **3-deoxyanthocyanins** lacking C-3 hydroxyl group are apigeninidin, luteolinidin (**9-80**), related 5- and 7- methoxylated derivatives and glycosides. These 3-deoxy forms have yellow to orange colour in acidic media and, unlike the red 3-hydroxy derivatives, they are stable even as aglycones. Red tricetinidin (**9-80**) is found in black tea infusions as a product of epigallocatechin gallate degradation.

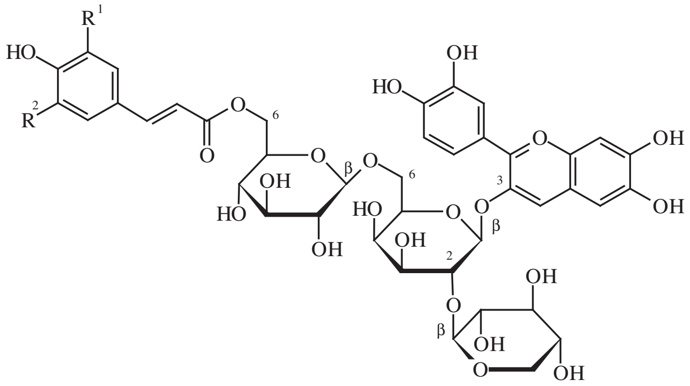


9-80, apigeninidin, $R^1 = R^2 = H$
luteolinidin, $R^1 = OH$, $R^2 = H$
triacetinidin, $R^1 = R^2 = OH$

Sugars bound in anthocyanins are often acylated by phenolic acids (*p*-coumaric, caffeic, ferulic and sinapic acids, less frequently by *p*-hydroxybenzoic acid), malonic acid and acetic acid. Acids are usually linked to the C-6 hydroxyl group of glucose and the C-4 hydroxyl group of rhamnose. 3-Glycosides are only rarely acylated. An exception is delphinidin-3-*O*-rutinoside-5,3′,5′-tri-*O*-glucoside, which contains four molecules of phenolic acids, some of which are bound to glucose occurring in other positions. An example of acylated pigments is (*E*)-petunidin-3-*O*-[6-*O*-(4-*O*-*p*-coumaroyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside, trivially called petanin (**9-81**), which is the characteristic pigment in the skin of red potato varieties. Other examples of acylated anthocyanins are pigments of black or purple carrots (*Daucus carota* ssp. *sativus* var. *atrorubens*). In recent years, many new varieties with an extremely high content of pigments



9-81, petanin



9-82, (*E*)-cyanidin-3-*O*-[2-*O*-β-D-xylopyranosyl-6-*O*-(4-*O*-*p*-coumaroyl)-β-D-glucopyranosyl]-β-D-galactopyranoside, $R^1 = R^2 = H$

have been cultivated. The anthocyanin content of several of these black carrot cultivars was reported to be in the range of up to 1750 mg/kg fresh weight. The dominating anthocyanins of these varieties are (*E*)-cyanidin-3-*O*-[2-*O*-β-D-xylopyranosyl-6-*O*-(4-*O*-*p*-coumaroyl)-β-D-glucopyranosyl]-β-D-galactopyranoside and related pigments derived from caffeic and sinapic acids (**9-82**).

Many other anthocyanins were known by trivial names in the past. Examples of these anthocyanins are cyanidin-3-*O*-glucoside or chrysanthemin, cyanidin-3-*O*-galactoside or idaein, cyanidin-3-*O*-rutinoside or ceracyanin, cyanidin-3-*O*-sambubioside or sambycyanin, cyanidin-3-*O*-(2-glucosyl)rutinoside or mecocyanin, delphinidin-3-*O*-glucoside or myrtillin, malvidin-3-*O*-glucoside or oenin and malvidin-3,5-di-*O*-glucoside or malvin.

9.4.1.2 Occurrence

Anthocyanins are found in many plant species located in cell vacuoles and stabilised by interactions of the type ion–ion with organic acids (malonic, malic and citric acids). The main sources of anthocyanin pigments that are used as food are fruits of plants of the grapevine family Vitaceae, which includes grapes, and of the rose family (Rosaceae), to which cherries, plums, raspberries, strawberries, blackberries, apples, pears and many other fruits belong. Other significant plants containing anthocyanine pigments belong to other plant families, such as eggplants (aubergines) and potato varieties with red skin (Solanaceae), black- and redcurrants and red varieties of gooseberries (Grossulariaceae), blueberries and cranberries (Ericaceae), olives (Oleaceae) and red cabbage, radishes and red varieties of other cruciferous vegetables (Brassicaceae). Lists of the main sources of anthocyanins and the individual main pigments of fruits, vegetables and other plant materials are given in Tables 9.11 and 9.12. The number of anthocyanins present in individual fruits and vegetables is variable and ranges from a few (e.g. in strawberries, blackberries and red cabbage) to more than ten different pigments (in blueberries and red varieties of grapes and maize). In addition to anthocyanins, many fruits and vegetables also contain yellow-to-orange carotenoids and anthoxanthins, green chlorophylls and other pigments, but never betalains, and the presence of these pigments often affects the resulting colour.

3-Deoxyanthocyanins are not commonly found in higher plants. They occur in higher quantities primarily in ferns and mosses and in sorghum cereal crops (such as *S. bicolor*, Poaceae). Apigeninidin and luteolinidin represent on average 36–50% of the total black and brown sorghum anthocyanins (2.8–4.3 g/kg).

Table 9.11 Main anthocyanins of selected fruits.

Fruits	Latin name	Main anthocyanins[a] [b]
Apples (skin)[c]	*Malus pumila*	**Cy-3-galactoside**, 3-glucoside, 3-xyloside, 3- and 7-arabinosideand acyl derivatives
Apricots (skin)	*Armeniaca vulgaris*	**Cy-3-glucoside**
Bilberries	*Vaccinium myrtillus*	**Df-3-galactoside** and 3-glucoside, **Cy-3-galactoside** and 3-glucoside, Pt, Pn and Mv glycosides
Blackberries	*Rubus* sp.	**Cy-3-glucoside**, 3-rutinoside, 3-xyloside, 3-(6-malonylglucoside), 3-[6-(3-hydroxy-3-methylglutaroyl]glucoside, Pg-3-glucoside
Blueberries	*Vaccinium myrtilloides*	**Mv-3-glucoside, 3-galactoside**, 3-arabinoside, **Pt-3-glucoside**, 3-arabinoside, 3-(malonoyl)glucoside, Cy-3-(malonoyl)glucoside, Dp-3-(malonoyl)glucoside
Cherries (sour)	*Cerasus vulgaris*	**Cy-3-(2-glucosyl)rutinoside**, **3-glucoside**, 3-rutinoside, 3-sophroside, 3-sambubioside, Pn-3-glucoside and other pigments
Cherries (sweet)[c]	*Cerasus avium*	**Cy-3-rutinoside**, **3-glucoside**, 3-sophoroside (some varieties), Pn-3-glucoside and 3-rutinoside, acyl derivatives
Chokeberries	*Aronia melanocarpa*	**Cy-3-galactoside**, **3-arabinoside**, 3-glucoside, 3-xyloside
Cornel berries	*Cornus mas*	**Cy-3-galactoside**, **Pg-3-galactoside** 3-rhamnosylgalactoside, Dp-3-galactoside
Cranberries	*Rhodococcus vitis-idaea*	**Cy-3-galactoside**, **3-arabinoside**, 3-glucoside and 3-xylosylglucoside, Df-3-glucoside, Mv-3,5-diglucoside
Currant (black)	*Ribes nigrum*	**Dp-3-rutinoside**, **3-glucoside**, **Cy-3-glucoside**, 3-rutinoside, Pt-3-rutinoside, Pn-3-rutinoside
Currant (red)	*Ribes silvestre*, syn. *R. rubrum*	**Cy-3-xylosylrutinoside**, **3-sambubioside**, **3-rutinoside**, 3-glucoside, 3-sophoroside, 3-glucosylrutinoside
Elderberries	*Sambucus nigra*	**Cy-3-glucoside**, **3-sambubioside**, 3-sambubioside-5-glucoside, 3,5-diglucoside
Figs (skin)	*Ficus carica*	**Cy-3-rutinoside**, 3-glucoside and 3,5-diglucoside, Pg-3-rutinoside
Gooseberries[c]	*Grossularia uva-crispa*	**Cy-3-rutinoside**, **3-glucoside**, 3-(6-*p*-coumaroyl)-glucoside, Pe and Pn-3-glucosides
Grapes[c]	*Vitis vinifera*	**Mv-3-glucoside**, **Mv-3-*p*-coumaroylglucoside** (in some varieties), **Mv-3-acetylglucoside** and Pt, Pe, Df and Cy glucosides and their acyl derivatives
	Vitis labrusca	Cy, **Dp**, Pt, Pn and **Mv-3-(*p*-coumaroyl)glucosides-5-glucosides**, 3,5-diglucosides, 3-*p*-coumaroylglucosides, **3-glucosides**
Lichee (skin)	*Litchi chinensis*	**Cy-3-rutinoside**, 3-galactoside, 3-glucoside, Pg-3,5-diglucoside, 3-glucoside
Mango	*Mangifera indica*	**Pn-3-galactoside**
Mulberries	*Morus nigra*	**Cy-3-glucoside**, **3-rutinoside**, 3-sophoroside, Pg-3-glucoside, 3-rutinoside,
Olives	*Olea europea*	**Cy-3-glucoside** and **3-rutinoside** and acyl derivatives
Oranges[c]	*Citrus sinensis*	**Cy-3-glucoside**, **3-(6-malonylglucoside)**, Dp and Pn-3-glucosides, pyranoanthocyanins
Passion fruit[c]	*Passiflora edulis*	**Pg-3-diglucoside**, **Dp-3-glucoside**
Peaches[c]	*Persica vulgaris*	**Cy-3-glucoside** and **3-rutinoside**
Pears (skin)[c]	*Prunus persica*	**Cy-3-galactoside**, **3-arabinoside**, 3-glucoside, 3-xyloside, Pe-3-galactoside and 3-rutinoside and acyl derivatives
Plums	*Prunus domestica*	**Cy-3-xyloside**, **3-rutinoside**, Pn-3-glucoside, 3-rutinoside
Pomegranate	*Punica granatum*	**Dp and Cy-3,5-diglucosides**, Pg-3,5-diglucoside, **Cy-3-glucoside**, Dp and Pg-3-glucoside
Raspberries	*Rubus idaeus*	**Cy-3-rutinoside**, **3-sophoroside**, **3-glucosylrutinosid**e, **3-glucoside**, Pg-3-glucosylrutinoside, 3-sophoroside, 3-glucoside, Mv-3-glucoside
Saskatoon berries	*Amelanchier alnifolia*	**Dp-3-glucoside**, **3-rutinoside**, **Mv-3-glucoside**, **Cy-3-glucoside**, 3-galactoside, 3-arabinoside, 3-xyloside
Sloeberries	*Prunus spinosa*	**Cy-3-rutinoside**, **3-glucoside**, **Pn-3-rutinoside**, 3-glucoside
Strawberries	*Fragaria* spp.	**Pg** and **Cy-3-glucosides**, their **succinates**, 3,5-diglucosides

[a]Cy = cyanidin, Pg = pelargonidin, Pn = peonidin, Dp = delphinidin, Pt = petunidin, Mv = malvidin.
[b]Components occurring in quantities higher than about 10% are highlighted in bold.
[c]Red varieties.

Table 9.12 Main anthocyanins of selected vegetables, cereals and other crops.

Material	Latin name	Main anthocyanins[a,b]
Vegetables		
Cabbage (leaves)[c]	*Brassica oleracea var. capitata*	**Cy-3-sophoroside-5-glucoside** (malonyl, ***p*-coumaroyl**, di-*p*-coumaroyl, feruloyl, diferuloyl, **sinapoyl** and disinapoyl esters)
Carrot (black)		**Cy-3-xylosyl(sinapoylglucosyl)galactosides, 3-xylosyl(feruloylglucosyl)galactosides**, other 3-xylosylgalactosides, Pn-3-xylosylgalactosides
Eggplant (skin)	*Solanum melongena*	**Dp-3-[4-(*p*-coumaroyl)rutinoside]-5-glucopyranoside**[d] (nasunin), **3-glucoside**, 3-rutinoside-5-glucoside, 3-rutinoside, , 3-*p*-coumaroylrutinoside, 3,5-diglucoside
Lettuce (leaves)[c]		**Cy-3-(3-malonoyl)glucoside, 3-(3-acetoyl)glucoside**, 3-(6-malonoyl)glucoside, 3-glucoside
Onion[c]	*Allium cepa*	**Cy-3-(malonoyl)glucoside-5-glucoside, 3-(3-acetoyl)glucoside**, glucoside, 3-galactoside, 3-laminaribioside, Pn-3-glucoside
Radishes[c] (skin)	*Raphanus sativus*	**Pg-3-(*p*-coumaroyl)diglucoside-5-(malonoyl)glucoside, 3-(feruloyl)diglucoside-5-(malonoyl)glucoside**, 3-(caffeoyl)diglucoside-5-(malonoyl)glucoside
Cereals		
Maize[e]	*Zea mays*	**Cy**, Pg and **Pn-3-glucosides** (free and acylated by malonic acid)
Blue barley[e]	*Hordeum vulgare*	**Pt** and **Cy-3-glucoside**
Black rice[e]	*Oryza sativa*	**Cy** and **Pn-3-glucoside**, Cy-diglucoside
Sorghum	*Sorghum bicolor*	**Ap, Lt** and Mv **glycosides**
Other crops		
Kidney beans[c]	*Phaseolus vulgaris*	**Pg-3-sambubioside**, 3-glucoside, Cy-3-glucoside,
Potatoes[c]	*Solanum tuberosum*	Pg, Cy, Pn, Dp, Pt and Mv-5-(*p*-coumaroyl)glucoside-3-rutinoside, Pg, Cy, Dp and Pt-3-rhamnosylglucosides, Pg and Pn-3-(*p*-coumaroyl)rutinosides-5-glucosides, 3-(feruloyl)rutinoside-5-glucoside
Roselle	*Hibiscus sabdariffa*	**Dp-3-sambubioside, Cy-3-sambubioside**
Sweet potato[f]	*Ipomoea batatas*	**Cy and Pn-3-caffeoylsophoroside-5-glucoside, Cy and Pn-3-p-hydroxybenzoylsophoroside-5-glucoside, Pn-caffeoyl-feruloylsophoroside-5-glucoside**

[a] Cy = cyanidin, Pg = pelargonidin, Pn = peonidin, Dp = delphinidin, Pt = petunidin, Mv=malvidin, Ap = apigeninidin, Lt = luteolinidin.
[b] Components occurring in quantities higher than about 10% are highlighted in bold.
[c] Red varieties.
[d] (*E*)- and (*Z*)-isomers.
[e] Black, blue and purple grains produced for making specialty foods or for use in ornamentation.
[f] Purple-fleshed.

9.4.1.2.1 Grapes and wine

European grapevine (*Vitis vinifera*, Vitaceae) varieties contain only 3-*O*-monoglucosides of anthocyanidins. North American and other species, such as river bank grape (*V. riparia*) and rock (mountain) grape (*V. rupestris*), fox grape (*V. labrusca*) and their hybrids with *V. vinifera* also contain the corresponding 3,5-di-*O*-glucosides and their acyl derivatives. The total amount of anthocyanins can range from about 300 to more than 7000 mg/kg fresh weight.

Distribution of anthocyanins in red grapes (*V. vinifera*) is highly variable and differs according to species, variety and a number of other variables. Determination of the individual pigments is also used in the chemotaxonomic classification of red grape varieties. The most abundant pigments are the 3-*O*-glucosides of malvidin and peonidin, as well as their 6′′-acetylated and *p*-coumaroylated derivatives. The 3-*O*-glycosides of petunidin, delphinidin and cyanidin are also widespread. For example, Cabernet Sauvignon contains 42.6% malvidin-3-glucoside, 20.5% malvidin-3-acetylglucoside, 10.0% delphinidin-3-glucoside, 6.4% malvidin-3-*p*-coumaroylglucoside, 6.1% petunidin-3-glucoside, 5.3% peonidin-3-glucoside, 2.5% delphinidin-3-acetylglucoside, 2.2% petunidin-3-acetylglucoside, 1.3% cyanidin-3-glucoside, 0.9% peonidin-3-acetylglucoside, 0.6% peonidin-3-*p*-coumaroylglucoside, 0.5% delphinidin-3-*p*-coumaroylglucoside, 0.4% petunidin-3-*p*-coumaroylglucoside, 0.1% malvidin-3-caffeoylglucoside

and smaller amounts of cyanidin-3-acetylglucoside and cyanidin-3-*p*-coumaroylglucoside. The important pigment in non-European grape species is malvidin-3,5-diglucoside.

Essentially the same pigments that are found in the grape skins from which they were extracted during fermentation are responsible for the young red wine colour. During wine maturation and aging, the colour of wine significantly changes. The amount of the original anthocyanins decreases, as they react with colorless flavan-3-ols, such as catechin, epicatechin and condensed tannins, to form oligomeric pigments and undergo other multiple reactions to form a heterogeneous mixture of typical darker and more stable red pigments, which are less sensitive to changes in pH (nucleophilic attacks of water, which results in the formation of carbinol pseudo base and subsequent loss of colour) or discoloration by sulfur dioxide. This is why mature wines are darker than young wines. The reaction mechanisms of formation of pigments in aged red wines are described in Section 9.4.1.5.9.

Polymerisation reactions in old red wines lead to the gradual formation of insoluble, brownish-red, high molecular weight condensation products called **phlobaphens**, which form sediments in wine. Certain other wine components, such as proteins, ascorbic acid, reducing sugars and metal ions can apparently be involved in their formation.

9.4.1.2.2 Apples and pears

Anthocyanin pigments responsible for the red colour of apple and pear peels are derived from cyanidin. The main pigment is cyanidin-3-galactoside (idaein), which represents about 94% of pigments in the Jonathan and 85% of all anthocyanins in the Red Delicious cultivars. Other pigments of these apple cultivars are cyanidin-3-arabinoside (10 and 4%) and cyanidin-3-glucoside (5 and 3%). Cyanidin-3-xyloside and acyl derivatives of the above listed anthocyanins are found in traces. The total amount of anthocyanins is about 100–200 mg/kg fresh weight.

Colourless flavan-3-ols or catechins or pale yellow flavonoids (flavonols, dihydrochalcones) and phenolic acids also act as co-pigments of anthocyanins. The main flavan-3-ols are (+)-catechin, (−)-epicatechin, (+)-gallocatechin and (−)-epigallocatechin. The main dihydrochalcone (its glycoside) occurring in all parts of the apple tree except for the mature fruit is phloretin-6-glucoside, known as phlorizin (**9-105**). The main phenolic acids of apples are *p*-coumaric, caffeic, ferulic and sinapic acids that are present mainly as depsides (called chlorogenic acids, which are isomers of caffeoylquinic, *p*-coumaroylquinic and furuloylquinic acids), glucose esters or glucosides.

Compared with other red fruits, the concentration of anthocyanins in pear peels is distinctly lower, ranging from 50 to 100 mg/kg of fresh weight. Pigments of red varieties of pears are derived mainly from cyanidin (unlike in apples, anthocyanidins of pears are not acylated by phenolic acids). The main pigments are cyanidin-3-galactoside, cyanidin-3-arabinoside and 3-rutinoside, in some cultivars peonidin-3-galactoside is also found.

Pears are rich in other flavonoids, mainly flavonols, flavan-3-ols and phenolic acids. The flavonols are largely represented by the kaempherol derivatives quercetin and isorhamnetin. The main components are quercetin-3-glucoside, 3,5-diglucoside and 7-xyloside, isorhamnetin-3-rutinoside, 3-glucoside, 3-rhamnosylgalactoside and 3-galactoside. Other components are malonyl glucosides of kaempherol, quercetin and isorhamnetin. As in apples, these glycosides act as anthocyanin co-pigments, which results in yellow-to-brown opacities and sediments in pear juices. The main flavan-3-ols present are (−)-epicatechin and (+)-catechin at about 10 and 2 mg/kg, respectively. The total content of phenolic acids is about 280 mg/kg, of which chlorogenic acid represents about 25%.

9.4.1.2.3 Sour cherries

The main pigments of sour cherries are anthocyanins derived from cyanidin. Their mean total content ranges from 350 to 820 mg/kg fresh weight, but may be even higher. For example, in the popular Morello variety the levels are 69–77% of the anthocyanin pigments cyanidin-3-(2-glucosyl)rutinoside (mecocyanin), 11–16% cyanidin-3-rutinoside, 3–15% cyanidin-3-glucoside and 1–3% cyanidin-3-sophoroside. The sour cherry variety Montmorency contains as the main pigment cyanidin-3-rutinoside (27%), followed by cyanidin-3-(2-glucosyl)rutinoside (25%), cyanidin-3-glucoside (19%) and cyanidin-3-sophoroside (16%). Minor pigments include cyanidin-3-sambubioside and 3-glucoside and peonidin-3-rutinoside (3%). Glycosides are accompanied by free aglycones cyanidin (7%) and peonidin (3%).

The yellow flavonoid pigments of sour cherries include kaempherol-3-rutinoside and 3-glucoside, followed by phenolic acids depsides, mainly derivatives of caffeoylquinic and *p*-coumaroylquinic acids that are accompanied by free acids.

9.4.1.2.4 Olives

The immature olive is grey–green, and later green to yellow–green; the fruit darkens on ripening and becomes violet and almost black. The main pigment of immature green olives is chlorophyll. Ripe purple to almost black olives contain anthocyanin pigments at a concentration of about 5000 mg/kg fresh weight in practically all cultivars. Besides anthocyanins, the pulp contains numerous anthoxanthins, phenolic acids and other polyphenols.

The main anthocyanins of ripe olives, which are concentrated mainly in the skin, are cyanidin-3-glucoside, cyanidin-3-rutinoside and their acylated derivatives. Contents of monoglycosides and diglycosides are roughly in the ratio 1 : 1 to 1 : 4. For example, the Spanish olive variety Manzanilla contains about 15% cyanidin-3-glucoside and other cyanidin glycosides, traces of 3-rutinoside, but 60% 3-rutinoside acylated by caffeic acid, traces of 3-glucosylrutinoside, 25% 3-(2-glucosyl)rutinoside and traces of this anthocyanine acylated by caffeic acid. In numerous other varieties further pigments were also found, such as cyanidin-3-(2-xylosyl)rutinoside, 3-rhamnosyldiglucoside acylated by caffeic acid and 3-glucoside acylated by *p*-coumaric acid. Particular anthocyanins derived from peonidin may also be present. In Greek olives anthocyanins derived from delphinidin are also present. Pigments of pickled black olives are formed in the enzymatic browning reactions.

9.4.1.2.5 Potatoes

There are more than 5000 varieties of potatoes in the world. Some potato varieties are distinguished only by a deeply coloured skin caused by anthocyanins; their flesh is the usual white or cream, which is due to the presence of carotenoid pigments. Others have coloured flesh. In South America, there are potatoes with a diversity of colours: purple, pink, orange and yellow, often with a contrasting colour around the eyes. The colour of the flesh is often similar to the skins. The so-called purple potatoes (or blue potatoes), even if not commonly found in our habitual diet, are now available on the European market. They have purple skin and flesh, which becomes blue once cooked. The colour of potatoes is determined by genetics. There are genes for purple pigments and genes that cover particular parts of the plant: flowers, leaves, skin, eyes and flesh.

The concentration of anthocyanins in potatoes varies over a wide range. The level of anthocyanins in skin tissue is quite high. However, the skin is such a small volume of the whole tuber that generally red-skinned white-fleshed potatoes contain about 15 mg anthocyanins per fresh weight. However, potatoes with anthocyanin in the flesh range from 150 to about 400–500 mg/kg fresh weight. The red-flesh potatoes contain predominantly acylated glucosides of pelargonidin. Purple-flesh potatoes have a more complex content of acylated glucosides of pelargonidin, petunidin, cyanidin and malvidin. For example, the purple-flesh variety Vitelotte Noire with the total anthocyanin content 486 mg/kg (fresh weight) contains as the main anthocyanin pigments (*E*)-malvidin-3-*O*-[6-*O*-(4-*O*-*p*-coumaroyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside (294 mg/kg) and (*E*)-petunidin-3-*O*-[6-*O*-(4-*O*-*p*-coumaroyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside]-5-*O*-β-D-glucopyranoside (125 mg/kg), known as petanin (**9-81**).

9.4.1.3 Use

Anthocyanins isolated from natural sources have been used as food colourings for more than 100 years, and for much longer in the form of various fruit juice concentrates. The disadvantage is that they only have the intense colour in solutions of pH < 3.5, so they are only suitable for acidic foods. Their importance as food colourings has increased in relation to consumers' growing interest in natural substances. Potential sources of these pigments are limited by the availability of the plant materials and overall economic conditions of their production, so only a few plant species may be used industrially.

The anthocyanin pigments most commonly used for food colouring are derived from grapes (skin or juice sediment), where the anthocyanin content ranges from 0.3 to 7.5 mg/kg. Historically, the oldest product of this type, produced in Italy since 1879, is called enocyanin (or enocianina). A rich source of anthocyanins is elderberries containing pigments at a concentration of of 2–10 g/kg fresh weight, or chokeberries (10 g/kg) that have similar composition of pigments. A very high content of anthocyanins is also found in blackberries (0.8–3.3 g/kg) that sometimes precipitate in juices. Other sources include red cabbage (containing 0.7–0.9 g/kg of anthocyanins), roselle calyces (15 g/kg dry matter), sometimes also sweet potatoes, red pulp oranges, leaves and seeds of red varieties of maize and locally also other products.

9.4.1.4 Biochemistry, physiology and nutrition

Flavonoids as well as stilbenes are synthesised from phenylalanine via cinnamic and *p*-coumaric acids. The extension of the chain by reaction with malonyl-CoA yields a tetraketide as an intermediate, the Claisen condensation of which produces chalcones. The basic flavonoids are naringenin chalcone (isosalipurpol) and isoliquiritigenin. Naringenin chalcone gives rise to flavanone (dihydroflavone) called naringenin (5,7,4′-trihydroxyflavanone). Hydroxylation and methylation of the skeleton creates additional hydroxyflavanones, which are, together with naringenin, precursors of all other flavonoids that arise by oxidation, reduction and dehydration reactions. Isoliquiritigenin is a precursor of isoflavonoids. Another chalcone (2,4,6,4′-tetrahydroxychalcone) is a precursor of quinochalcones.

The biological properties of anthocyanins have not yet been explored extensively. Their toxicity and mutagenicity were examined in relation to their use as food additives, but were not proved or were very low, so that anthocyanin pigments are generally allowed for food colouring, and in most countries there are no limits on their use. Very low toxicity was observed even in their metal complexes with Al(III) and Sn(II) ions. Apart from imparting colour to food raw materials and foods, anthocyanins also have an array of health-promoting benefits, as they can protect against a variety of oxidants through a number of mechanisms.

9.4.1.5 Properties and reactions

From the technological perspective, the most important property of anthocyanins is colour and its stability, which is usually relatively low. The main factors affecting the colour stability of anthocyanins are the structure of the molecule, presence of certain enzymes, pH, temperature, presence of oxygen and exposure to radiation. Anthocyanins may produce various coloured or colourless reaction products in reactions with other food components, such as, for example, ascorbic acid, sulfur dioxide, other phenols and metal ions.

9.4.1.5.1 Effect of pH

In aqueous solutions, anthocyanins are transformed into various products of different colour. Depending on pH, the following five different aglycone structures are in equilibrium (Figure 9.9):

- red flavylium cation
- colourless carbinol pseudo base
- purplish-red neutral quinoid base
- blue quinoid base
- yellow chalcones.

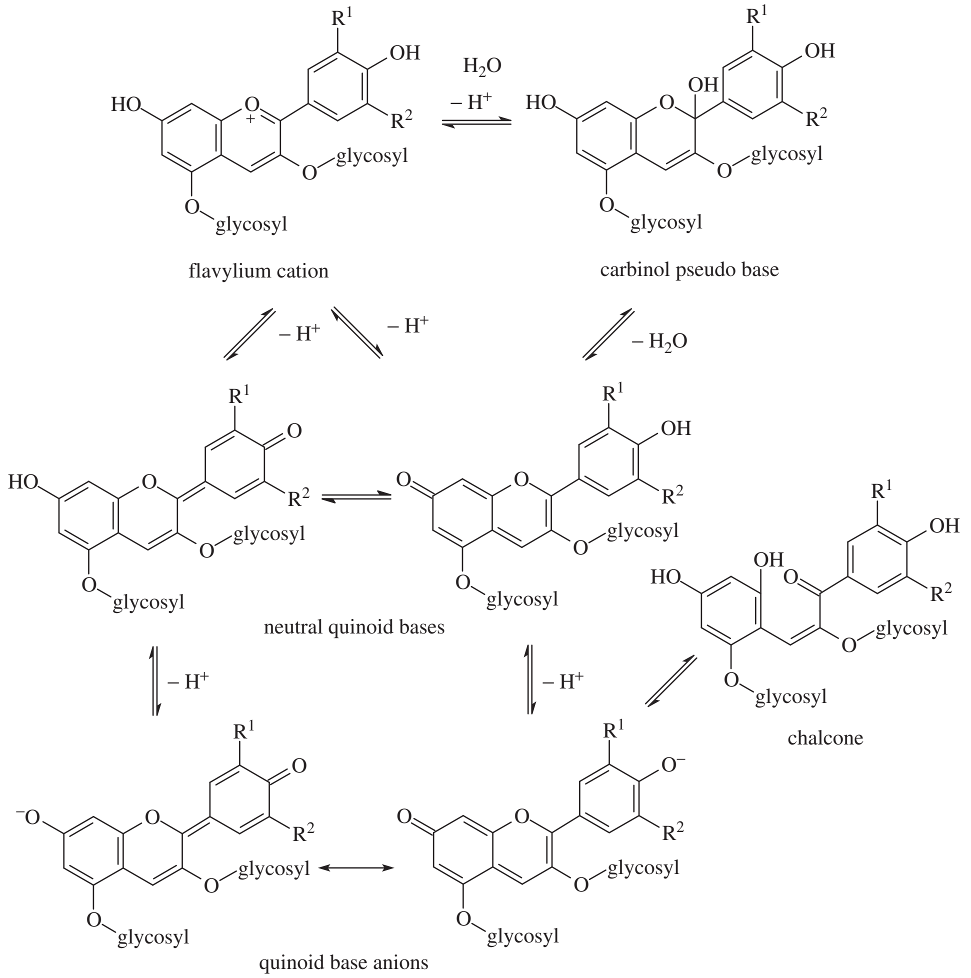


Figure 9.9 Transformations of anthocyanins according to pH (R^1 and R^2 = H, OH, OCH_3 or *O*-glycosyl).

In solutions of pH 1.0 and lower, anthocyanins exist solely as red-coloured flavylium salts. When increasing pH, the equilibrium shifts in favour of colourless carbinol pseudo base and the red colour fades. Around the range of pH values of 4.0 to 4.5, anthocyanins are completely colourless. Another increase in pH is manifested by the purplish-red colour, which is caused by formation of a neutral quinoid base that requires the presence of free hydroxyl groups on one of C-5, C-7 or C-4′ carbons. In solutions of pH 7 a blue coloured quinoid base is formed. After some time or following an increase in pH value, a gradual decrease of blue colour intensity occurs as a result of yellow chalcone formation. If the solution is acidified to around pH 1.0, the blue quinoid and colourless carbinol bases are converted back into red flavylium cations. The transformation of chalcones is slower and not quantitative.

In processed fruit and vegetables, the situation is more complex. Anthocyanins in plants (pH of from 2.5 to 7.5) occur as a purplish-red neutral quinoid base, but in food products they may be in media of different pH. However, they are mostly stabilised by inter co-pigmentation (interactions with other flavonoids) or intra co-pigmentation (acylated forms), or by interactions with other food components. Many products therefore retain their original colour or possess some discoloration. In products stored long-term (such as strawberry jams, olives) oligomers are then formed with colour similar to the original colour of the anthocyanins, while the original pigments may be absent altogether.

9.4.1.5.2 Effect of structure

The colour of non-acylated and monoacylated anthocyanins in acidic media is mainly dependent on the number and type of aglycone (anthocyanidin) substituents. Anthocyanidins with a higher number of hydroxyl groups tend to have a blue tint, and methoxyl derivatives have a red tint. Derivatives with a higher number of hydroxyl groups are less stable, and their stability increases with a growing number of methoxyl groups. Glycosides and their acyl derivatives (anthocyanins) generally have a red–blue colour and are more stable than the corresponding aglycones (anthocyanidins). During storage, heat treatment and exposure to light, diglycosides are more stable than monoglycosides. The type of bound sugar has some influence on the stability of the pigments.

The presence of one or more acyl groups stabilises anthocyanins (due to the so-called intramolecular co-pigmentation), and their

reaction with water in neutral or weakly acidic environments does not lead to the formation of a colourless quinoid base, but preferably to a blue quinoid base. These pigments are less sensitive to changes in pH, and colour is stable in weakly acidic and neutral media.

9.4.1.5.3 *Effect of temperature*

Like most chemical reactions, the stability of anthocyanins and the rate of their degradation are affected by temperature, and also depend on the pH value, their structure, the presence of oxygen and the possibility of entering into reactions with other components of the system.

Degradation of anthocyanins in the absence of oxygen, at pH ranging from 2.0 to 4.5, is virtually independent of pH and usually takes place under aerobic and anaerobic conditions as a first-order reaction. In the presence of oxygen, 3-glycosides of anthocyanidins show the highest stability at elevated temperatures in the pH range of 1.8 to 2.0, and 3,5-diglycosides are stable at pH 4.0–5.0. Most anthocyanins somewhat paradoxically exhibit increased stability at the elevated temperatures used in the processing of fruits and vegetables. This phenomenon is explained by the protective effect of various components of the system, and by condensation of monomers that leads to the formation of more stable oligomeric pigments, whose content increases with temperature and storage time. Oligomeric pigments are important colour carriers, especially of stored fruit juices and red wines.

The mechanism of anthocyanin degradation reactions depends not only on temperature, but also on the structure of substances. Decomposition reactions of 3-glycosides of anthocyanidins are shown in Figure 9.10. The major products are glycosides of the corresponding chalcones. Hydrolysis of glycosidic bonds in chalcones yields α-diketones (1,2-diketones). Subsequent reactions of the primary degradation products (most of them are colourless compounds) produce brown-coloured polymeric products. Somewhat different is the decomposition mechanism of 3,5-diglycosides of anthocyanidins, where diglycosides of coumarins arise as the main reaction products (Figure 9.11).

9.4.1.5.4 *Enzymes*

Loss of colour of anthocyanins may also be caused by enzymatic reactions catalysed by two groups of enzymes:

- glycosidases, which hydrolyse the glycosidic bond to form sugar and anthocyanin aglycone (anthocyanidin), which is unstable and spontaneously transformes into colourless products;
- polyphenol oxidases that also act in the enzymatic browning reactions.

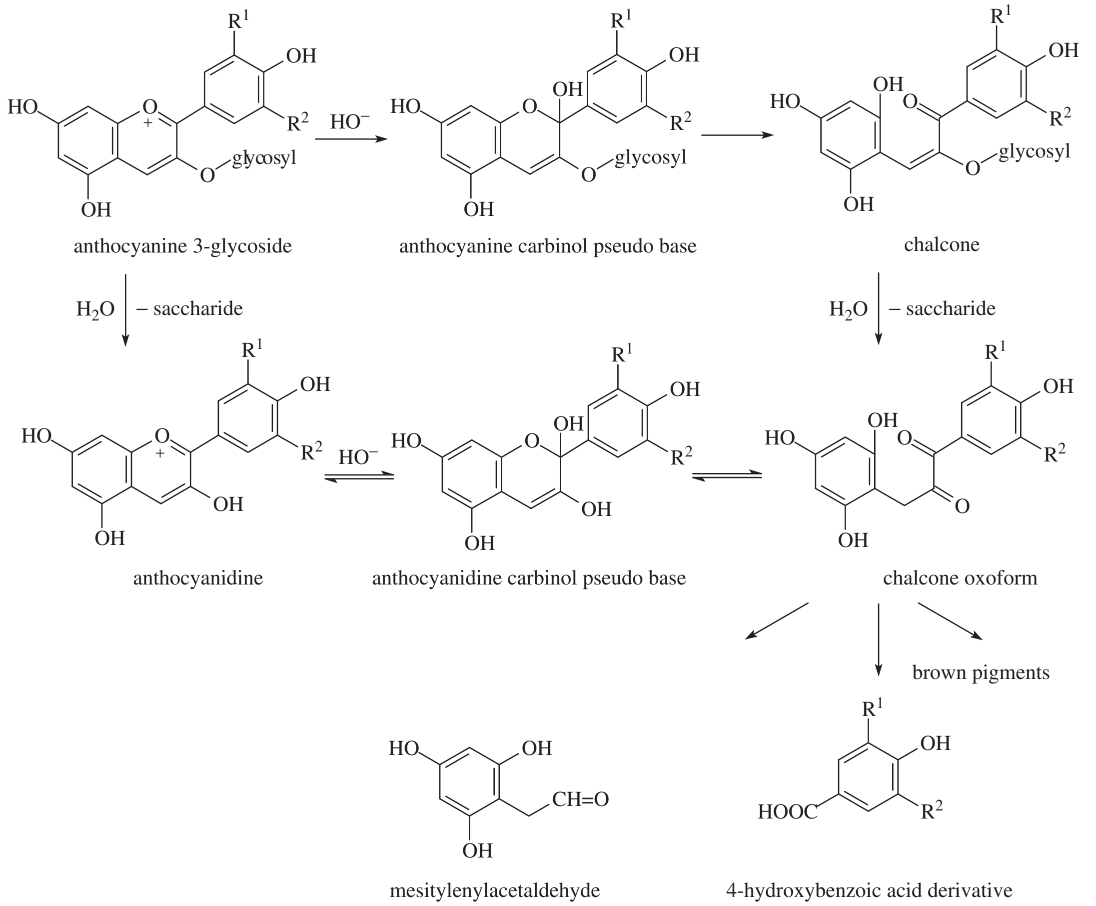


Figure 9.10 Degradation of 3-glycosides of anthocyanidins (R^1 and R^2 = H, OH, OCH_3 or *O*-glycosyl).

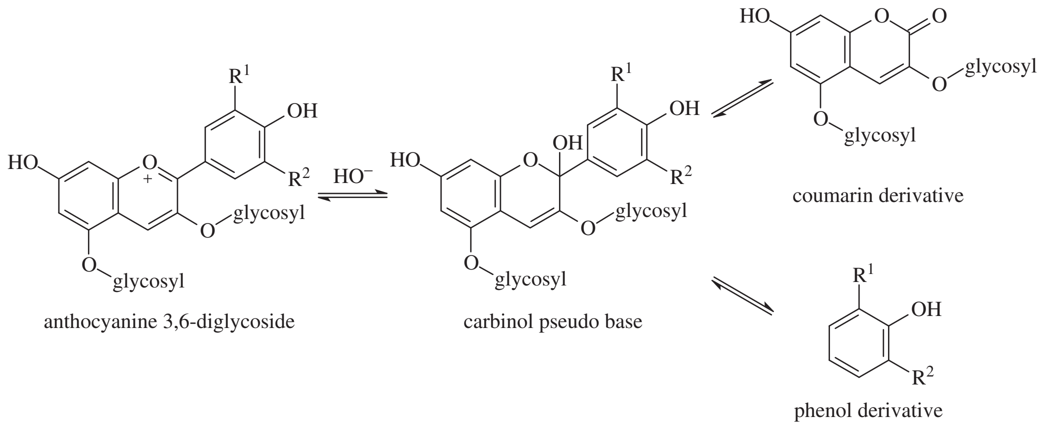
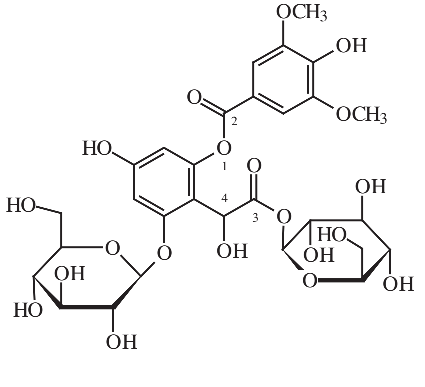


Figure 9.11 Degradation of 3,5-diglycosides of anthocyanidins (R^1 and R^2 = H, OH, OCH_3 or *O*-glycosyl).

9.4.1.5.5 Oxygen and peroxides

Atmospheric oxygen oxidises anthocyanins to colourless or brown coloured products directly or through other labile compounds that are preferentially oxidised by oxygen (such as ascorbic acid). Degradation of anthocyanins that is induced by ascorbic acid proceeds indirectly via hydrogen peroxide, which arises by ascorbic acid oxidation. For example, the anthocyanin pigment malvin (malvidin-3,5-diglucoside), found in flowers of the mallow genus *Malva* (*M. sylvestris*, Malvaceae) and in a variety of fruits and flowers, is oxidised by hydrogen peroxide under the opening of the heterocyclic ring between C-2 and C-3, which yields a colourless substance malvone (**9-83**). A hydrogen atom may replace the hydroxyl group at C-4. On alkaline hydrolysis, malvone affords syringic acid. A similar degradation of anthocyanin hirsutin (hirsutidin-3,5-diglucoside) affords colourless hirsulone.



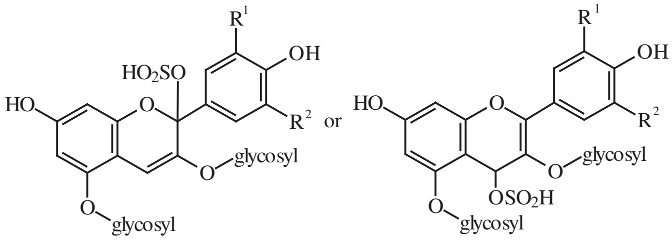
9-83, malvone

9.4.1.5.6 Radiation

Anthocyanins are unstable when exposed to visible, ultraviolet or ionising radiation. Decomposition takes place mainly as photooxidation. Anthocyanins substituted at C-5 hydroxyl group, which are fluorescent compounds, are more sensitive to photochemical degradation in comparison with C-5 unsubstituted anthocyanins.

9.4.1.5.7 Sulfur dioxide

The natural anthocyanin pigments form adducts with bisulfite ion. The bisulfite ion forms a bond to position C-2 or C-4 of the flavylium nucleus, which decolorises the pigment and simultaneously stabilises the glycosidic bond at position C-3 (**9-84**). The adducts are stable at pH about 3, but acidification to pH < 1 and heating leads to reversal of the reaction with quantitative recovery of the pigment. Such adducts are therefore likely to be likewise unstable under gastric conditions.



9-84, reaction products of anthocyanins with bisulfites

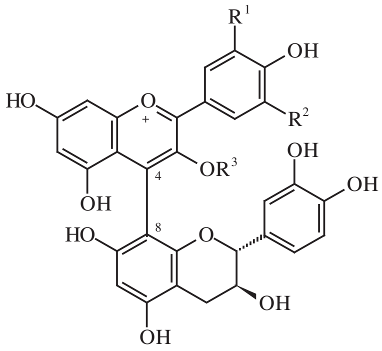
9.4.1.5.8 Sugars and their degradation products

Sugar concentrations higher than 20% (found in jams and similar products) have a stabilising effect on the colour of anthocyanins, mainly due to decreased water activity. Degradation of anthocyanins is accelerated in the presence of sugar degradation products, especially by furan-2-carbaldehyde and 5-hydroxymethylfuran-2-carbaldehyde that produce complex brown-coloured condensation products with anthocyanins.

9.4.1.5.9 Transformed pigments in red wines

Anthocyanins are relatively unstable and undergo numerous chemical reactions in beverages like red wines during fermentation processes, maturation and aging. The monomeric anthocyanins extracted from the grape skin are a crucial contribution to the colour of young red wine. Their intramolecular interactions and intermolecular interactions with other wine constituents and especially

phenolic compounds can further enhance the colour of wine. One of these reactions is based on the interaction (called co-pigmentation) of anthocyanin with flavan-3-ols (procyanidins, called co-pigments) through which a complex of these two compounds initially arises and subsequently the final pigments, which are dimers linked by covalent bonds. An example is a dimer of anthocyanin and (+)-catechin linked by a C4→C8 bond, which is formed in the nucleophilic attack of the phloroglucinol ring of catechin on the electron deficient C-4 position of anthocyanin (**9-85**). This dimer has a red colour similar to the colour of anthocyanin, even though the co-pigment is colourless flavan-3-ol. Another possibility for the formation of more stable pigments in red wines is the reaction of anthocyanins with colourless flavan-3-ols through their nucleophilic C-8 position (less likely through the C-6 position) mediated by aldehydes, such as the fermentation product acetaldehyde, which leads to coloured anthocyanin-alkylene-(epi)catechin conjugates (Figure 9.12). Acetaldehyde may also mediate analogous self-condensation of anthocyanins. The other aldehydes arising in wines as fermentation products and intermediates (such as propionaldehyde and isobutyraldehyde) or aldehydes extracted from oak wood during wine aging (such as benzaldehyde and cinnamaldehyde derivatives) may be involved in similar reactions leading to various anthocyanin-alkylene-(epi)catechin dimers. Some aldehydes, such



9-85, anthocyanin-catechin dimer

as methylglyoxal, furan-2-carbaldehyde, 5-hydroxymethylfuran-2-carbaldehyde may react directly with flavan-3-ols yielding alkylene-linked dimers. Their oxidation and dehydration yields xanthylium-type pigments (Figure 9.13). Cinnamic aldehydes, such as coniferaldehyde (R^1 = H, R^2 = OCH_3) and sinapaldehyde (R^1 = R^2 = OCH_3) produce, via alkylene-linked dimers, orange pigments called **oaklins** (Figure 9.13).

During maturation and aging, the concentration of monomeric anthocyanins decreases, while numerous more complex and stable anthocyanin-derived pigments are formed. One of the most

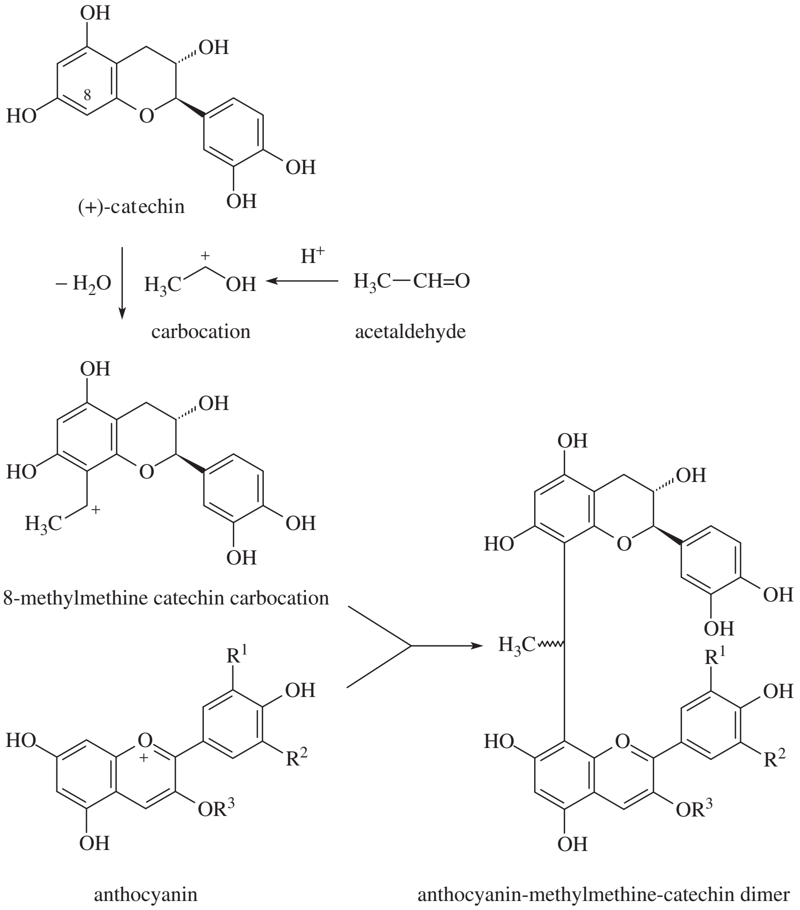


Figure 9.12 Reactions of anthocyanins with flavan-3-ols and aldehydes.

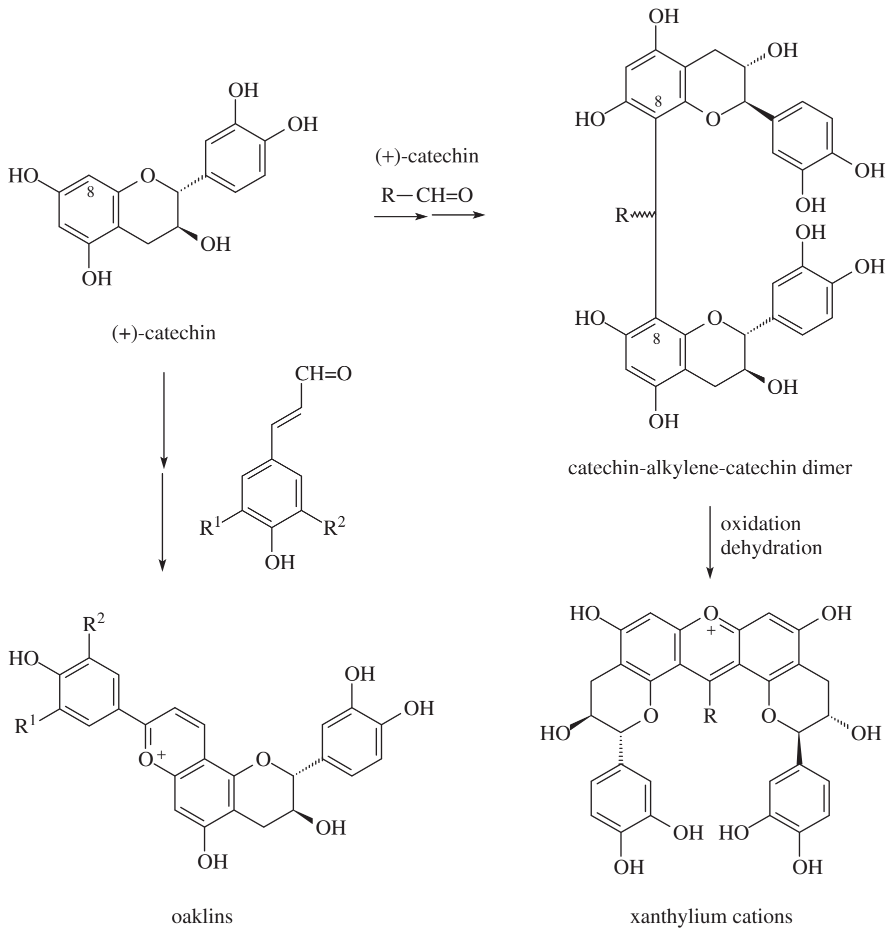


Figure 9.13 Reactions of aldehydes with flavan-3-ols.

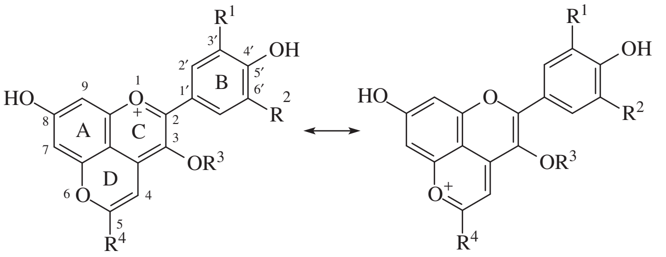


Figure 9.14 Basic structure of pyranoanthocyanins: (R^1, R^2 = H, OH or CH_3, R^3 = sugar residue).

interesting groups of anthocyanin reaction products in red wines is **pyranoanthocyanins** (Figure 9.14) possessing an additional pyran ring structure between C-4 and the hydroxyl group at C-5 of anthocyanin, which seems to be responsible for relatively higher stability of pyrananthocyanins (e.g. their resistance to pH changes and sulfur dioxide bleaching). The newly formed pyran ring is either unsubstituted or substituted at C-5 by COOH (in carboxypyranoanthocyanins), CH_3 (in methylpyranoanthocyanins), phenols (in pyranoanthocyanin-phenols) or flavanols (in pyranoanthocyanin-flavanols).

Among the pyranoanthocyanins bearing different moieties of fermentation products, the major and most important group occurring naturally in red wines is **vitisins**. The substituted carboxypyranoanthocyanins (A-type vitisins with carboxyl group at C-5) arise from the cycloaddition reaction of pyruvic acid to an anthocyanin moiety (Figure 9.15), while unsubstituted pyranoanthocyanins (B-type vitisins) are formed by reaction of anthocyanins with acetaldehyde as minor pigments. Vitisin A and vitisin B are derived from the main pigment of red wines malvidin-3-*O*-glucoside. Also identified in aged wines are yellow methylpyranoanthocyanins (with a methyl group at C-5) derived from malvidin-3-*O*-glucoside, which form by cycloaddition reaction of anthocyanins with acetoacetic acid followed by decarboxylation and isomerisation. For example, vitisin A derived from malvidin-3-*O*-glucoside and its acylated forms (acetyl and *p*-coumaroyl glucosides) have been identified in aged Port red wine.

More recently a group of neutral non-oxonium pyranoanthocyanins occurring in aged Porto red wine and named as **oxovitisins A** have been identified. Oxovitisins A arise by hydration of vitisins A, decarboxylation of the formed intermediate and isomerisation of the decarboxylation product (Figure 9.16).

In red wines, 4-hydroxycinnamic acids (such as *p*-coumaric, caffeic, ferulic and syringic acids) and their decarboxylation

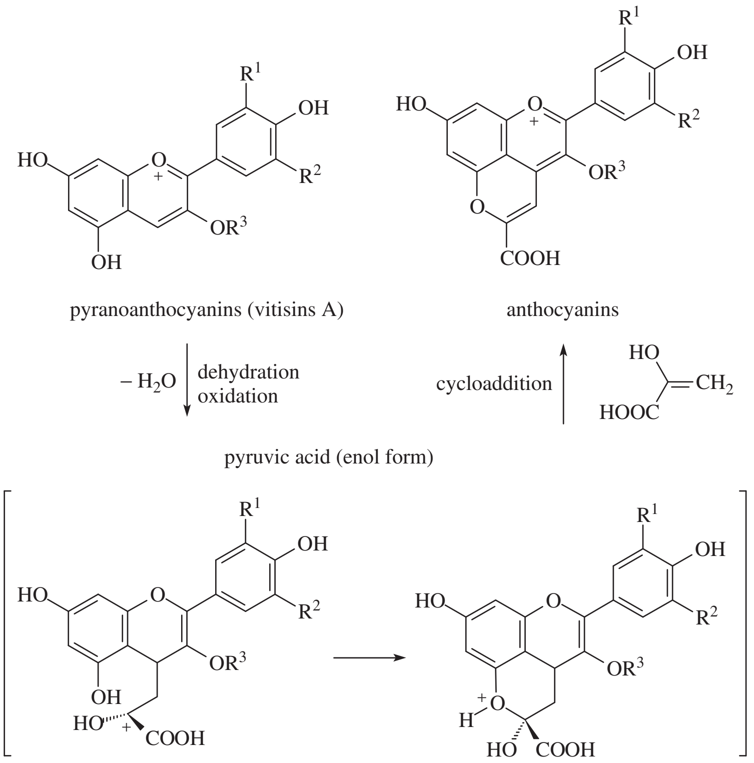


Figure 9.15 Reaction of anthocyanins with pyruvic acid and formation of A-type vitisins: (R^1, R^2 = H, OH or CH_3, R^3 = sugar residue).

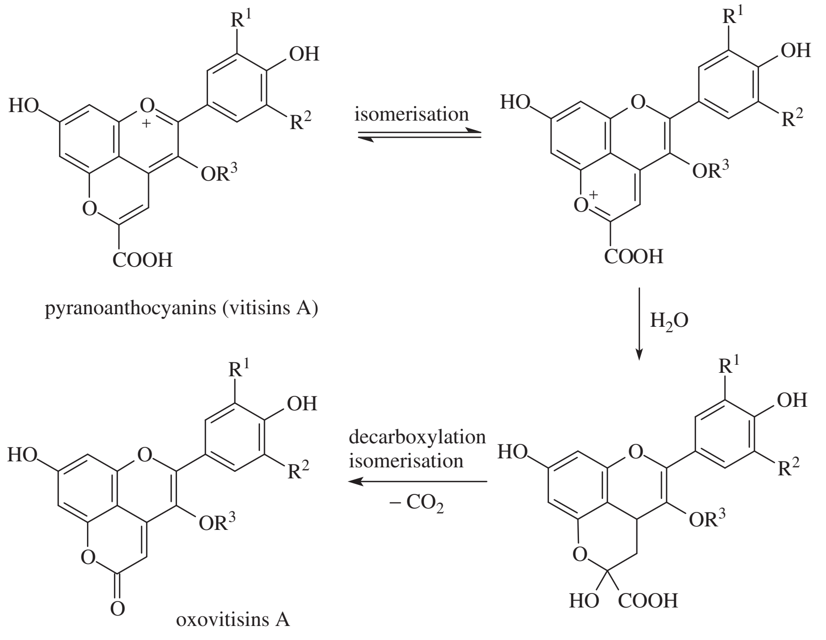
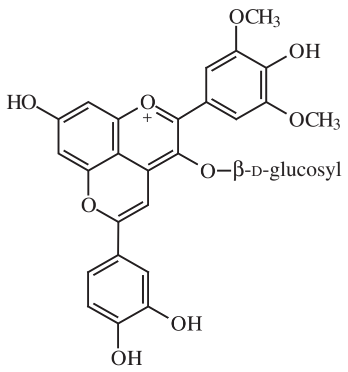


Figure 9.16 Formation of A-type oxovitisins from A-type vitisins: (R^1, R^2 = H, OH or CH_3, R^3 = sugar residue).

products 4-vinylphenols (such as 4-vinylphenol, 4-vinylcatechol, 4-vinylguaiacol and 4-vinylsyringol) can react with free anthocyanins to yield pyranoanthocyanin-phenols (also known as hydroxyphenyl-pyranoanthocyanins), some of which are named **pinotins**, since they were first isolated from *Vitis vinifera* cv. Pinotage wines. The formation of the best known representative of this family of pigments pinotin A (**9-86**) is analogous to the formation of A-type vitisins (Figure 9.15). The nucleophilic C-2-position of caffeic acid attacks the electrophilic C-4 position of malvidin-3-*O*-glucoside to form an intermediate carbonium ion, in which the reaction of C-5 hydroxyl of the anthocyanin moiety forms a pyran ring.

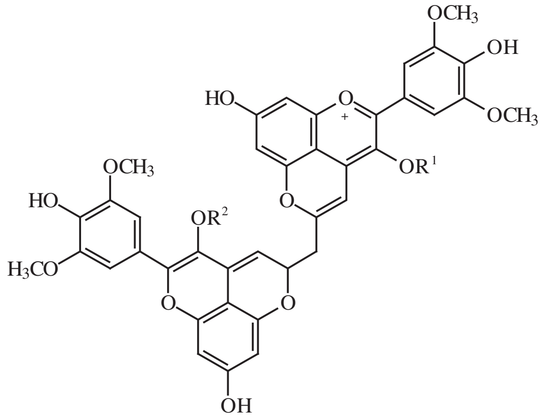


9-86, pinotin A

Orange pyranoanthocyanin-flavanols (also known as flavanyl-pyranoanthocyanins or vinylflavanol-pyranoanthocyanins) isolated from Port red wine contain a pyroanthocyanin moiety linked directly to flavan-3-ols or procyanidins. These pigments arise in wines from the reaction of anthocyanins with 8-vinylflavan-3-ols (such as 8-vinylcatechin) or 8-vinylprocyanidins (such as 8-vinylprocyanidin B_3) mediated by acetaldehyde as in the case of anthocyanin-alkylene-(epi)catechin conjugates (Figure 9.12). 8-Vinylflavanols are not present in grapes, but they have been proposed to result from the dehydration of the flavanol–ethanol adducts formed by reaction of flavanols with acetaldehyde. Examples of pyranoanthocyanin-flavanols generated from malvidin-3-*O*-glucoside and (+)-catechin and malvidin-3-*O*-glucoside and procyanidin B_3 are given in formulae **9-87**. In addition to pyranoanthocyanin-flavan-3-ol monomers and pyranoanthocyanin-procyanidin dimers more polymerised pyranoanthocyanin-flavanols (up to tetramers) have been found in some red table wines and Port red wine.

Related blue–violet vinylpyranoanthocyanins (also known as flavanyl/phenyl-vinylpyranoanthocyanins) named **portisins** are produced in Port red wine during aging. A-Type portisins arise from the reaction between 8-vinylflavanols and carboxypyranan-thocyanins (vitisins A), followed by the elimination of formic acid, which gives rise to the vinyl bridge (Figure 9.17). B-Type portisins arise from vitisins A and 4-hydroxycinnamic acids. The corresponding reaction mechanism is analogous to that of portisins A, but it involves further decarboxylation of the intermediate.

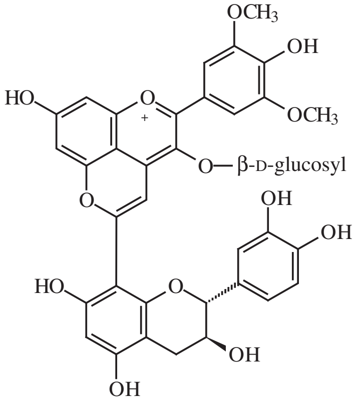
A relatively new class of turquoise coloured pyranoanthocyanin dimers derived from delphinidine glycosides was identified recently in aged Port wine and shown to be formed by reactions of carboxypyranoanthocyanin with methyl-pyranoanthocyanin. The structure of the identified pyranoanthocyanin methine dimer is given in formula **9-88**.



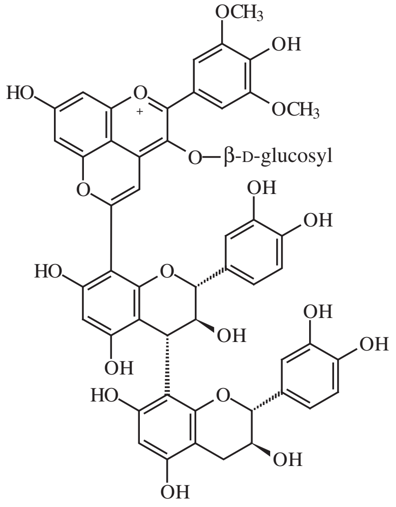
9-88, pyranoanthocyanin dimer

9.4.1.5.10 Transformed pigments in fruits and vegetables

Some of the pigments occurring in aged wines are similarly found in anthocyanin-rich fruits and vegetables. Three pyranoanthocyanins, the 3-glucosides of 5-carboxypyranodelphinidin,



malvidin-3-*O*-glucoside-(+)-catechin



malvidin-3-*O*-glucoside-procyanidin B_3

9-87, pyranoanthocyanin-flavanols

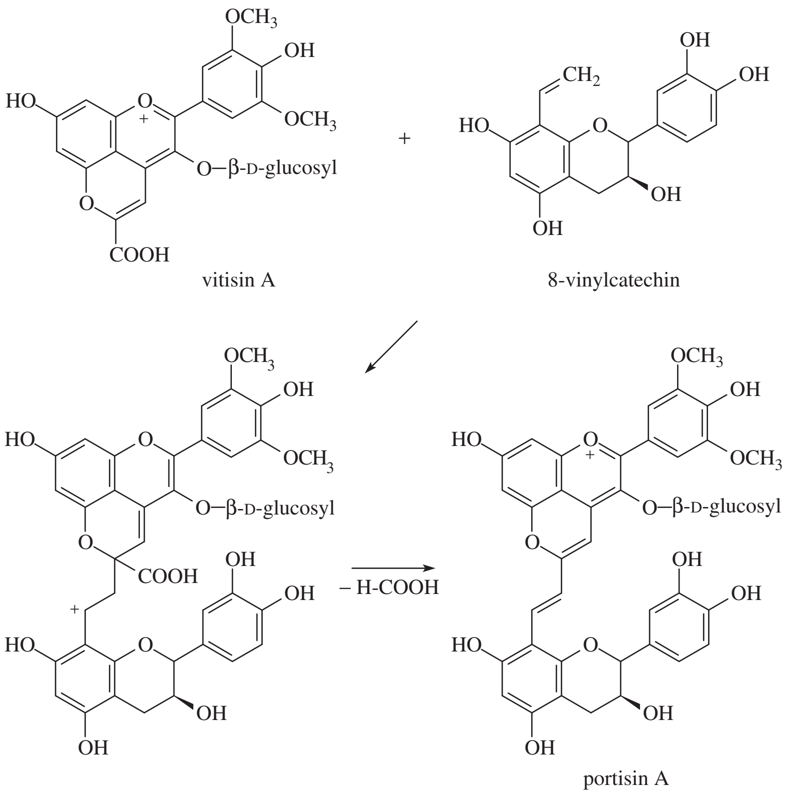


Figure 9.17 Formation of portisin A.

5-carboxypyranopetunidin and 5-carboxypyranomalvidin, were produced in an extract of black beans (*Phaseolus vulgaris*) fortified with pyruvic acid. Pyranoanthocyanin-phenols derived from cyanidin-3-*O*-glucoside and 4-vinylphenol, 4-vinylcatechol, 4-vinylguaiacol and 4-vinylsyringol were identified in blood orange juice. Anthocyanin-flavanol reaction products (derived from cyanidin and delphinidin) were also found in blackcurrants and strawberries. Vinylcatechol adducts of cyanidin (derived from 4-vinylphenol, 4-vinylcatechol and 4-vinylguaiacol) were isolated from black carrots.

9.4.1.5.11 Other reactions

Anthocyanin with the structure of *o*-diphenols form complexes with metals (such as Al, K, Fe, Cu, Ca and Sn) that can stabilise the colour of products, but also may cause unwanted discolorations. For example, complexes with tin formed in cans may change the red colour of fruits (such as strawberries) to purple.

9.4.2 Other flavonoids

Other flavonoids that are usually pale yellow-to-dark yellow pigments include flavanones, flavanonols, flavones, flavonols, chalcones, aurones and isoflavones. The most important food pigments are flavones and flavonols. However, with few exceptions, these flavonoid substances are not used as food colourings. Previously, some of these compounds were ranked among the so-called bioflavonoids (see Section 5.15) for their biological effects.

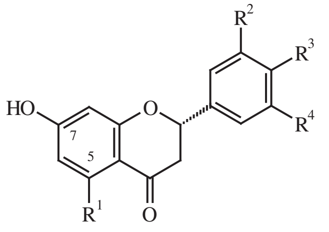
Flavonoids usually exist in the form of *O*-glycosides, which contain either free or acylated D-glucose, L-rhamnose, D-galactose, L-arabinose, D-xylose, D-apiose or D-glucuronic acid. Sugars are bound at positions C-7, C-5, C-4′, C-3′, but mostly through the hydroxyl group attached to the C-7 carbon. In addition to *O*-glycosides, flavonoids are quite often found as *C*-glycosides (mainly derived from flavones and flavonols), in which glucose is bound by the C–C bond in positions C-6 or C-8 of flavonoid molecules.

Hydrolysis of glycosides in the manufacturing of fruits and vegetables may in some cases (in acidic media and in particular at higher temperatures) lead to increased concentrations of aglycones. Most flavonoids in foods are involved in enzymatic browning reactions. The ability to bind heavy metals, along with the ability to terminate the radical oxidation reactions, gives flavonoids many antioxidant properties. However, metal complexes of flavonoids in foods sometimes cause unwanted discolorations.

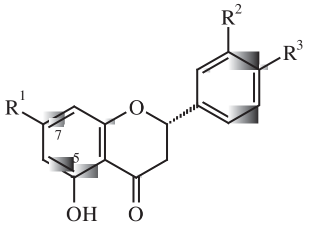
9.4.2.1 Flavanones

Colourless to pale yellow flavanones are widespread in foods, but are not important as food pigments. At higher concentrations they are found only in citrus fruits. The main components are glycosides derived from (2*S*)-5,7-dihydroxyflavanones, which differ in substituents of the ring C (**9-89** and **9-90**).

The most important flavanone aglycones are hesperetin (5,7,3′-trihydroxy-4′-methoxyflavanone) and naringenin (5,7,4′-trihydroxyflavanone). Hesperetin is the major aglycone occurring



9-89, flavanones
liquiritigenin, $R^1 = H$, $R^2 = H$, $R^3 = OH$, $R^4 = H$
butin, $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$
pinocembrin, $R^1 = OH$, $R^2 = H$, $R^3 = H$, $R^4 = H$
naringenin, $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = H$
eriodyctiol, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$
dihydrotricetin, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$



9-90, flavanone methoxyderivatives
sakuranetin, $R^1 = OCH_3$, $R^2 = H$, $R^3 = OH$
sterubin, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = OH$
isosakuranetin, $R^1 = OH$, $R^2 = H$, $R^3 = OCH_3$
hesperetin, $R^1 = OH$, $R^2 = OH$, $R^3 = OCH_3$
homoeriodyctiol, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$

in oranges and lemons. Its contents in juices is about 1000 mg/l. Grapefruits contain naringenin as the main glycoside component.

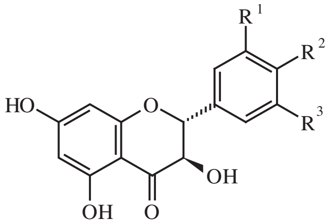
Common glycosides of citrus and other plant materials are sakuranin (sakuranetin-5-glucoside), narirutin (naringenin-7-rutinoside, also known as neoponcirin), naringin (naringenin-7-neohesperidoside), eriodictin (eriodictyol-7-rhamnoside), ericitrin (eriodictyol-7-rutinoside), poncirin (isosakuranetin-7-neohesperidoside), hesperidin (hesperetin-7-rutinoside) and neohesperidin (hesperetin-7-neohesperidoside). In citrus fruits, flavanone glycosides are located mainly in the albedo. The main glycoside of oranges is hesperidin; the dominating glycoside (up to 90% of total glycosides) in grapefruits is naringin. Their contents in growing fruits increase till full ripeness and then remain constant at the level of 1–6 g per fruit.

Oranges contain only rutinosides (such as hesperidin and narirutin), and grapefruits contain rutinosides and neohesperidosides. The presence of naringin in orange juice therefore indicates the presence of grapefruit juice. Glycoside naringin (naringenin-7-neohesperidoside)occurring in grapefruits, neohesperidin (hesperitin-7-neohesperidoside) found in bitter bigarade oranges (*Citrus aurantium* var. *amara*, Rutaceae) and all other flavanone neohesperidosides are intensely bitter substance, unlike corresponding rutinosides and glucosides.

Liquiritin (liquiritigenin-4′-glucoside) occurs in licorice (*Glycyrrhiza glabra*, Fabaceae), sakuranin and prunin (naringenin-7-glucoside) in plums (*Prunus* spp., Rosaceae), pinocembrin glycosides occur in legumes, pyracanthoside (eriodictyol-7-glucoside) in firethorn (*Pyracantha coccinea*, Rosaceae). Butin and its 7,3′-diglucoside butrin are components of the tree *Butea monosperma* with orange–red flowers native to tropical and sub-tropical parts of the Indian Subcontinent and Southeast Asia that are used for colouring food, in medical products and to treat liver diseases. Prenylated flavanones (see Section 10.4) occur in hop cones.

9.4.2.2 Flavanonols

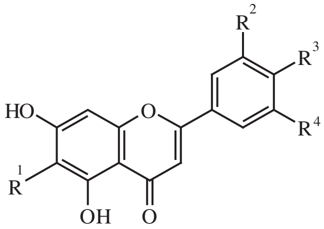
Flavanonols and their glycosides are not very significant flavonoids, because they do not occur at higher concentrations in food materials. An example of a flavonol is taxifolin (dihydroquercetin, **9-91**), which occurs in larger quantities in peanuts and as a components of pollen and along with other flavanonols is quite a common component of other plants.



9-91, flavanonols
pinobanksin, $R^1 = R^2 = R^3 = H$
aromadendrin (dihydrokaempherol), $R^1 = R^3 = H$, $R^2 = OH$
taxifolin (dihydroquercetin), $R^1 = R^2 = OH$, $R^3 = H$
ampelopsin (dihydromyricetin), $R^1 = R^2 = R^3 = OH$

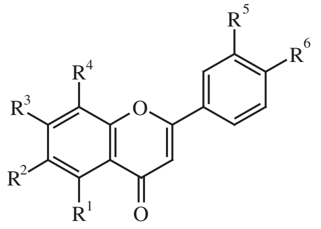
9.4.2.3 Flavones

Flavones are, together with flavonols, the most widespread yellow pigments of plants. Typical compounds in foods are flavones substituted at C-5 and C-7, less often in the position C-6 of ring A and at C-4′ of ring B. If the substituent occurs in position C-4′, then the carbons C-3′ and C-5′ and rarely the carbon C-2′ are also often substituted. Common substituents are hydroxyl and methoxyl groups. Particularly frequent flavones are apigenin and luteolin (**9-92**), less often, tricetin and other flavones occur.



9-92, flavones
chrysin, $R^1 = H$, $R^2 = H$, $R^3 = H$, $R^4 = H$
apigenin, $R^1 = H$, $R^2 = H$, $R^3 = OH$, $R^4 = H$
luteolin, $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$
tricetin, $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$
baikalein, $R^1 = OH$, $R^2 = H$, $R^3 = H$, $R^4 = H$
scutellarein, $R^1 = OH$, $R^2 = H$, $R^3 = OH$, $R^4 = H$

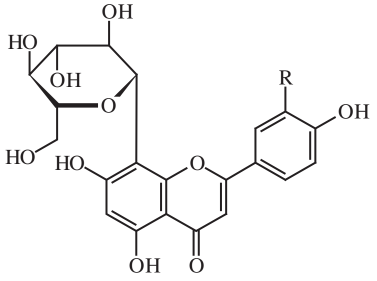
Examples of flavones with methylated hydroxyl groups in ring A are hispidulin, nepetin and cirsiliol, while acacetin, diosmetin and chrysoeriol have methoxyl groups in ring B. Methoxyl groups in both rings A and B have limocitrin, tangeretin, nobiletin and sinensetin (**9-93**), which occur in different types of citrus fruits, especially in peel, together with a number of other hydroxylated polymethoxyflavones and other flavonoids.



9-93, flavone methoxy derivatives
hispidulin, $R^4 = R^5 = H$, $R^1 = R^3 = R^6 = OH$, $R^2 = OCH_3$
nepetin, $R^4 = H$, $R^1 = R^3 = R^5 = R^6 = OH$, $R^2 = OCH_3$
cirsiliol, $R^4 = H$, $R^1 = R^5 = R^6 = OH$, $R^2 = R^3 = OCH_3$
acacetin, $R^2 = R^4 = R^5 = H$, $R^1 = R^3 = OH$, $R^6 = OCH_3$
diosmetin, $R^2 = R^4 = H$, $R^1 = R^3 = R^5 = OH$, $R^6 = OCH_3$
chrysoeriol, $R^2 = R^4 = H$, $R^1 = R^3 = R^6 = OH$, $R^5 = OCH_3$
limocitrin, $R^2 = H$, $R^1 = R^3 = R^6 = OH$, $R^4 = R^5 = OCH_3$
tangeretin, $R^5 = H$, $R^1 = R^2 = R^3 = R^4 = R^6 = OCH_3$
nobiletin, $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = OCH_3$
sinensetin, $R^4 = H$, $R^1 = R^2 = R^3 = R^5 = R^6 = OCH_3$

Sugars (mainly D-glucose, D-galactose and L-rhamnose) in *O*-glycosides of flavones are preferentially bound to the hydroxyl group in position C-7. Other *O*-glycosides and *C*-glycosides are rare. For example, curly mint (*Mentha crispa*, Lamiaceae) contains the glycoside known as diosmin (diosmetin-7-rutinoside). Unlike in anthocyanins, flavones do not occur as *O*-diglycosides.

The most common *C*-glycosides are vitexin (8-*C*-glucosylapigenin) and orientin (8-*C*-glucosylluteolin, **9-94**) that occur, for example, in rice bran and many fruits. Further common *C*-glycosides are isovitexin (6-*C*-glucosylapigenin) and isoorientin (6-*C*-glucosylluteolin). Orientin and isoorientin, the main components of fermented roiboos tea (*Aspalathus linearis*, Fabaceae), show antioxidant properties. Vitexin, isovitexin, flavone *C*-glycoside chafuroside A (with a potent anti-inflammatory activity) and its regioisomer chafuroside B (**9-95**) have been isolated from oolong tea. For example, *C*-glycoside, known as schaftoside (apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-α-L-arabinopyranoside), is found in figs, and its presence may be used to detect fig juice in other fruit juices, for example, in grape juices.



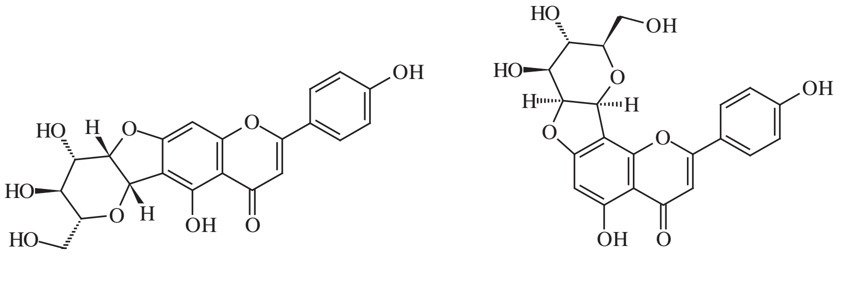
9-94, flavone *C*-glucosides
vitexin, R = H
orientin, R = OH

A special separate group of about 60 pigments derived from flavones are **biflavonoids**. Biflavonoids are usually dimers of apigenin, with molecules linked by bonds C8→C8, C8→C3′ or C8-C4′ that have no importance as food pigments. Amentoflavone (**9-96**) and other biflavonyls, for example, occur in large quantities in the leaves of ginkgo, also known as the maidenhair tree (*Ginkgo biloba*, Gingoaceae). Leaf extracts containing flavonoids and terpenoids (so called ginkgolides and bilobalides) are used in pharmacy for problems with blood circulation in some parts of the body, and to strengthen the memory, improve concentration and to treat Alzheimer's disease.

9.4.2.4 Flavonols

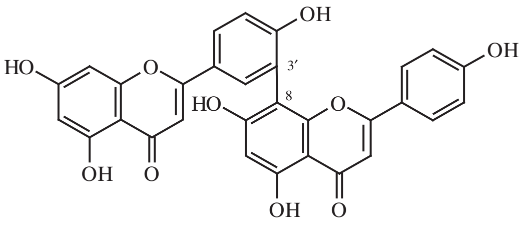
Flavonols are, together with flavones, an important group of yellow plant pigments. All major flavonols occurring in foods have hydroxyl groups in positions C-3, C-5, C-7 and C-4 and mutually differ by substitutions at positions C-3′ and C-5′. Almost universal flavonols are kaempherol, quercetin and myricetin (**9-97**), which occur mainly as glycosides and as co-pigments accompanying anthocyanins. A particularly high content of quercetin (2.5–6.5%) is found in the dry outer skin of red onion cultivars.

An atypical flavonol is morin (**9-97**) occurring in the leaves of white mulberry (*Morus alba*, *Moraceae*). It is derived from kaempherol, but contains an additional hydroxyl group at position C-2′, as well as another flavonol datiscetin present in the leaves and roots of bastard hemp (*Datisca cannabina*, Datiscaceae), the

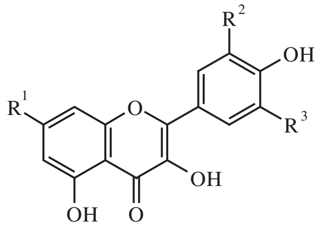


chafuroside A　　chafuroside B

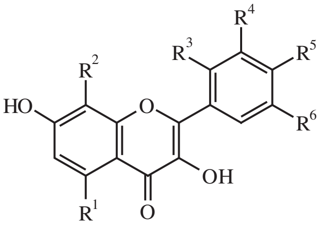
9-95, chafurosides



9-96, amentoflavone



9-98, flavonol methoxy derivatives
rhamnetin, $R^1 = OCH_3$, $R^2 = OH$, $R^3 = H$
isorhamnetin, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = H$
laricitrin, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OH$
syringetin, $R^1 = OH$, $R^2 = OCH_3$, $R^3 = OCH_3$



9-97, flavonols
galangin, $R^2 = R^3 = R^4 = R^5 = R^6 = H$, $R^1 = OH$
datiscetin, $R^2 = R^4 = R^5 = R^6 = H$, $R^1 = R^3 = OH$
kaempherol, $R^2 = R^3 = R^4 = R^6 = H$, $R^1 = R^5 = OH$
quercetin, $R^2 = R^3 = R^6 = H$, $R^1 = R^4 = R^5 = OH$
myricetin, $R^2 = H$, $R^1 = R^4 = R^5 = R^6 = OH$
physetin, $R^1 = R^2 = R^3 = R^6 = H$, $R^4 = R^5 = OH$
robinetin, $R^1 = R^2 = R^3 = H$, $R^4 = R^5 = R^6 = OH$
morin, $R^1 = R^2 = R^4 = R^6 = H$, $R^3 = R^5 = OH$
herbacetin, $R^3 = R^4 = R^6 = H$, $R^1 = R^2 = R^5 = OH$
gosypetin, $R^3 = R^6 = H$, $R^1 = R^2 = R^4 = R^5 = OH$

Asiatic herb looking like hemp, which is used for its yellow pigment and also as a laxative. In the roots, datiscetin is accompanied by galangin. Herbacetin and gosypetin have an additional hydroxyl group at C-8. Examples of flavonol methyl ethers are rhamnetein, isorhamnetin and other substances (**9-98**). Isorhamnetin occurs as 3-β-rutinoside (narcissin) in citrus and other fruits.

Free aglycones are found in relatively small quantities, and the main forms are flavonol glycosides. Flavonol glycosides are mainly 3-glycosides and less often the 7-glycosides. The most widespread compounds are glycosides derived from quercetin and kaempherol; glycosides of myricetin are less common. Trivial names of common glycosides derived from quercetin are given in Table 9.13. Normally, however, the systematic names of flavonoids are favoured.

A generally widespread glycoside of many plants is rutin. Avicularin and isoquercitrin occur, for example, in blueberries (*Vaccinium myrtillus*, *Ericaceae*); spiraein, together with rutin, is a constituent of onion (onions are a primary source of dietary quercetin glycosides in the Western diet), chestnuts (seeds of horse chestnut, *Aesculus hippocastanum*, Sapindaceae) and the flowers of some plants (such as elm meadowsweet, *Filipendula ulmaria*, Rosaceae). A relatively common glycoside is myricitrin (myricetin-3-rhamnoside), while in cotton plants (*Gossypium* spp., Malvaceae) occur populnin (kaempherol-7-glucoside), quercimeritrin, herbacitrin (herbacetin-7-glucosidea) and gosypitrin (gosypetin-7-glucoside). For example, apples contain, as major glycosides, quercetin-3-glucoside and 3-galactoside (depending on the variety and other factors, they are found in levels of about 0.01– 0.95 g/kg), 3-rhamnoside (0.01–0.37 g/kg), 3-arabinoside (0.02–0.78 g/kg), 3-xyloside (0.03–0.25 g/kg) and 3-rutinoside (0.01–0.12 g/kg). In smaller amounts, apples contain 3-glucosides of kaempherol and myricetin. Elderberries (*Sambucus nigra*, Adoxaceae) contain quercetin-3-rutinoside that is missing in strawberries, quercetin-3-glycoside (trisaccharide containing two molecules of L-rhamnose and one molecule of D-glucose) occurs in blackcurrants, but not in redcurrants. The presence of these flavonoids in fruit juices can be used to detect of adulteration, such as the replacement of individual types of berries in fruit juices and addition of low-cost fruits in fruit spreads produced from strawberries, raspberries and blackcurrants.

Flavonols and their glycosides are found in tea (*Camellia sinensis*, Theaceae) in larger quantities than they are in fruits. The main components of all teas are 3-glucosides, 3-galactosides and 3-rutinosides of flavonols, 3-rhamnoside of quercetin and 3-rhamnodiglucosides and 3-glucorhamnogalactosides of quercetin and kaempherol), which significantly contribute to the bitter taste of tea infusions. Black teas usually contain 0.4–1.7% glycosides, green tea 1.5–1.7% glycosides and instant teas from 2.6 to 3.1% glycosides in dry matter.

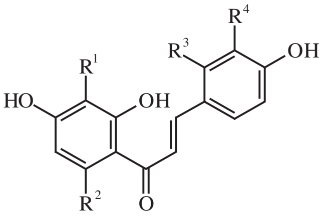
Table 9.13 Trivial and systematic names of selected quercetin glycosides.

Trivial name	Systematic name	Trivial name	Systematic name
Avicularin	quercetin-3-α-L-arabinofuranoside	Spiraein (spiraeoside)	quercetin-4′-β-D-glucopyranoside
Reinoutrin	quercetin-3-β-D-xylopyranoside	Hyperin (hyperoside)	quercetin-3-β-D-galactopyranoside
Isoquercitrin	quercetin-3-β-D-glucopyranoside	Quercitrin	quercetin-3-α-L-rhamnopyranoside
Quercimeritrin	quercetin-7-β-D-glucopyranoside	Rutin	quercetin-3-β-rutinoside

Rutin and several other flavonoid glycosides exhibit antioxidant properties and affect the flexibility and permeability of blood capillaries. Rutin (formerly vitamin P) is thus used in pharmaceutical preparations and food supplements. Together with other substances called bioflavonoids (see Section 5.15), rutin increases levels of ascorbic acid in various animal organs, either by protection against oxidation catalysed by metal ions, or by increased ascorbic acid utilisation in the body. Natural sources of ascorbic acid containing flavonoids (such as rosehips with a considerable amount of rutin) are thus more effective than synthetic vitamin C. A complex of rutin with iron causes dark discoloration of asparagus in tins, and the stannous complex of rutin is yellow.

9.4.2.5 Chalcones

Chalcones, dihydrochalcones and aurones are not particularly important components of plant food materials, but they occur as notable pigments of flowers of many ornamental plants, such as the common snapdragon (*Antirrhinum* spp., Scrophulariaceae) (Asteraceae), cosmos (*Cosmos* spp.) and dahlia (*Dahlia* spp.) from the Asteraceae family. Chalcones are also pigments of legume seeds (**9-99**) and woods. Prenylated chalcones are found in hop cones (**10-98**).



9-99, chalcones
isoliquiritigenin, $R^1 = R^2 = R^3 = R^4 = H$
butein, $R^1 = R^2 = R^3 = H$, $R^4 = OH$
marein, $R^1 = R^4 = OH$, $R^2 = R^3 = H$
okanin, $R^1 = R^3 = R^4 = OH$, $R^2 = H$
chalconaringenin, $R^1 = R^3 = R^4 = H$, $R^2 = OH$

Isoliquiritigenin, for example, occurs in soybeans as 4′-*O*-β-D-glucopyranoside. The most common chalcone is butein. Its 4-glucoside is called coreopsin and 4,3′-diglucoside is isobutrin. These glycosides are found as pigments of tropical flowers of the legume plant *Butea monosperma* (Fabaceae), which are used locally for food colouring (see Section 9.4.2.1). Marein and okanin are pigments in the central part of the flowers of tickseed, also known as calliopsis (*Coreopsis* spp., Asteraceae). The pigment absorbs UV radiation (unlike the carotenoid pigments in the other parts of the flowers) and is an attractant for bees and other insects that pollinate the flowers. Chalconaringenin is the predominant compound in tomatoes. Its concentration is 9–182 mg/kg, which comprises 35 to 71% of the total flavonoid content.

Chalcones always contain a hydroxyl group at C-2, which comes from the pyrane ring C of flavanones, which produce chalcones in alkaline medium (Figure 9.18). Under acidic conditions, especially with heating, the opposite reaction proceeds – conversion of chalcones into flavanones. For example, naringenin chalcone gives rise to naringenin; hesperidin arises from hesperidin chalcone and isobutrin from butrin. These reactions are related to the enzymatic reactions of chalcone biosynthesis, which catalyses chalcone-flavanone isomerase.

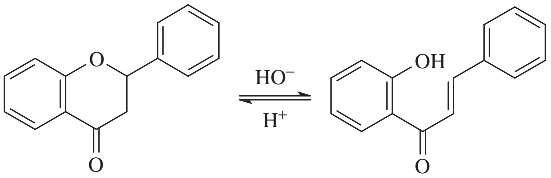
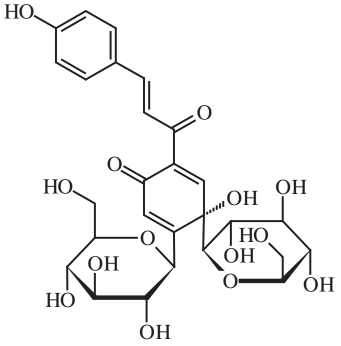


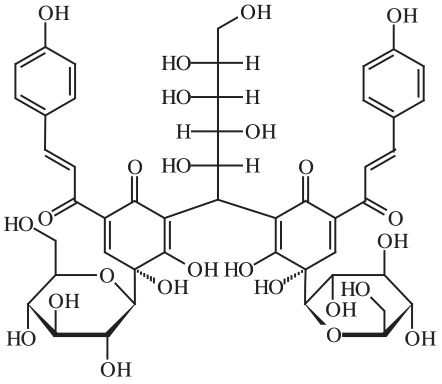
Figure 9.18 Interconversion of flavanones and chalcones.

9.4.2.5.1 Quinochalcones

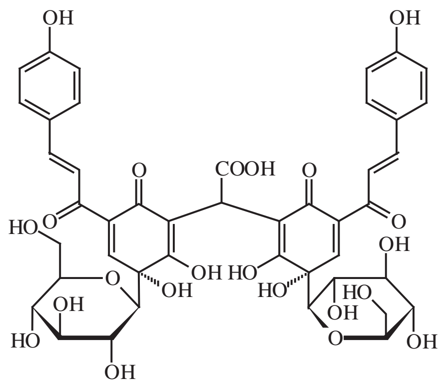
Carthamin (carthemon) is a yellow-to-red, water-soluble pigment derived from safflower flowers (*Carthamus tinctorius*, Asteraceae). Safflower seeds are used for making edible oil, which is used in cooking. The pigment is a mixture of three coloured compounds, red carthamin, safflor yellow A, safflor yellow B and also contains small amounts of their precursors. The precursor of carthamin in flowers is 2,4,6,4′-tetrahydroxychalcone, which is glycosylated at first to safflor yellow A (**9-100**) and then further to safflor yellow B (**9-101**), which gives on oxidation yellow precarthamin (**9-102**), decarboxylation and further oxidation of which yields carthamin (**9-103**). Isomerisation of carthamin in an acidic environment yields yellow isocarthamin (**9-104**). Hydrolysis of carthamin gives glucose and two aglycones – flavanones carthamidin and isocarthamidin.



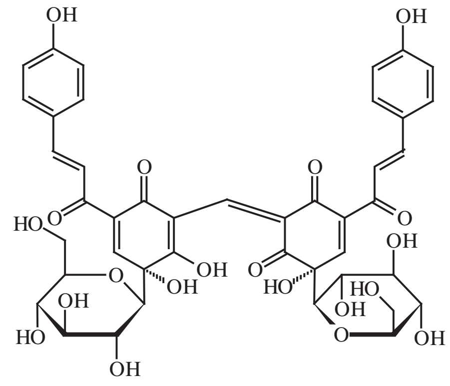
9-100, safflor yellow A



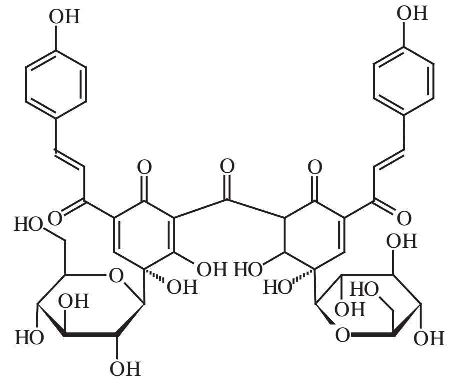
9-101, safflor yellow B



9-102, precarthamin



9-103, carthamin

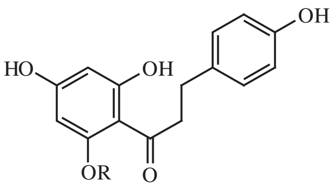


9-104, isocarthamin

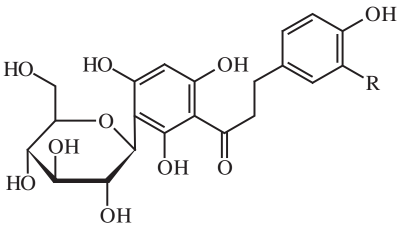
Carthamin was used as a wool dye in ancient times and is now the only chalcone type pigment recommended in some countries for food colouring. As a food additive, carthamin is known as Natural Red 26. Its properties are not yet well known, but it may become a promising food colouring, for example for yoghurt and other dairy products.

9.4.2.6 Dihydrochalcones

An example of natural dihydrochalcones is phloretin (**9-105**). The most famous glycoside of phloretin, phloretin-6-*O*-β-D-glucoside, is called phlorizin. Its occurrence is practically limited to the botanical genus *Malus* (apple), where phlorizin functions as an inhibitor of seed germination. In mammals, phlorizin causes glycosuria (glucose excretion in the urine). It has a bitter taste, like naringin. In small quantities, phlorizin is also found in apples and apple products (at a level of 0.1–22 mg/kg dry matter in the skin, from 0.03 to 0.3 mg/kg in the flesh and from 0.01 to 0.4 mg/kg in the juices), where it is accompanied by a small amount of 6-*O*-xyloglucoside. The presence of these characteristic flavonoids of apples may be used to detect the addition of apple juice to other fruit juices. Phloretin-3′5′-di-*C*-β-D-glucopyranoside is the first *C*-glycoside identified in tomatoes and also the first dihydrochalcone from *Solanum* species. In tomatoes, its amount ranges from 2 to 15 mg/kg. High levels of the dihydrochalcone *C*-glucosides aspalathin and nothofagin (**9-106**) with antioxidant activities are present in the rooibos plant (*Aspalathus linearis*, Fabaceae). Aqueous spray-dried extracts of fermented rooibos usually contain up to 0.5% aspalathin. Fermented rooibos is the most common form used by the food industry as a herbal tea or in products such iced teas and yoghurt. The fermentation process, necessary for development of the characteristic rooibos flavour and colour, however, greatly reduces the aspalathin content of the plant material and leads to the formation of flavones isoorientin and orientin as major products.



9-105, dihydrochalcones
phloretin, R = H
phlorizin, R = *β*-D-glucosyl



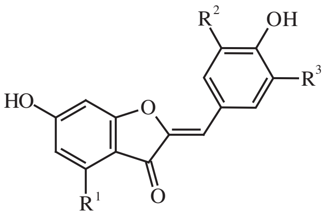
9-106, nothofagin, R = H
aspalathin, R = OH

Enzymatic and acid hydrolysis of phlorizin releases the aglycone phloretin, alkaline hydrolysis yields 4-hydroxyphenylpropionic acid and β-D-glucopyranosideside of phloroglucinol (1,3,5-trihydroxybenzene), which is called phlorin. It has been shown that phlorin is present in peel of citrus fruits. In species and varieties of oranges, phlorin was found in juices and peel extracts with a mean of 22 and 492 mg/l, respectively, while for grapefruits, means were 108 mg/l in juices and 982 mg/l for peel extracts. In contrast, phlorin was not found in mandarin and clementine juices except for a few mandarin varieties (30–33 mg/l).

Dihydrochalcones prepared semi-synthetically by reduction of flavanones neohesperidin and naringin have an intensely sweet taste. Neohesperidin dihydrochalcone is used as a sweetener (see Section 11.3.2.1.2).

9.4.2.7 Aurones

Just as with chalcones and dihydrochalcones, aurones, 2-benzylidenebenzo-2*H*-furan-3-ones, are likewise not very important pigments of food raw materials. For example, hispidol has been found in soybeans as 6-*O*-β-D-glucoside. Aurones, however, are widespread in various flowers where they play a significant role in their pigmentation. Sulfuretin (**9-107**) is a pigment of dahlia (*Dahlia* spp., Asteraceae) flowers, aureusidin occurs in flowers of the common snapdragon (*Antirrhinum majus*, Plantaginaceae), where it occurs as 6-*O*-β-D-glucoside (called aureusin) together with 4-*O*-β-D-glucoside of aureusidin (cernuoside) and 6-*O*-β-D-glucoside of bracteatin.



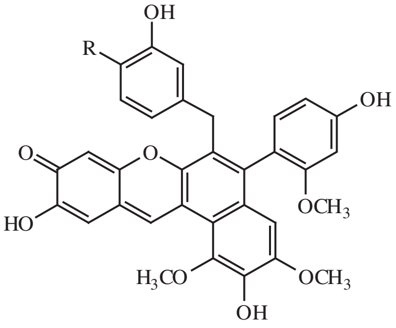
9-107, aurones
hispidol, $R^1 = H$, $R^2 = H$, $R^3 = H$
sulfuretin, $R^1 = H$, $R^2 = OH$, $R^3 = H$
aureusidin, $R^1 = OH$, $R^2 = OH$, $R^3 = H$
bracteatin, $R^1 = OH$, $R^2 = OH$, $R^3 = OH$

9.4.2.8 Isoflavones

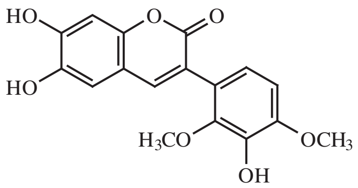
Isoflavones have been found in higher concentrations only in the legume family of plants (Fabaceae) and occur in significant amounts only in soybeans and soya bean products. Isoflavones exhibit oestrogenic activity, but also further toxic effects, and are often classified, together with other active compounds, as phytoestrogens (see Section 10.4).

9.4.2.9 Santalins

The lipophilic red pigments santalins, santalin A and santalin B (**9-108**), are (along with some other related compounds, such as the coumarin derivative santalin AC (**9-109**) and yellow isoflavone 3′,4′,5-trihydroxy-7-methoxyisoflavone) components of the slightly fragrant red sandalwood (red sanders) and red sandalwood extracts obtained from *Pterocarpus santalinus* (Fabaceae), which is grown mainly in India. Biosynthesis of santalins is based on anthocyanins and lignans. Their main use has previously been in dyeing wool and in folk medicine for antiseptic properties. Currently, red sandalwood is used for colouring tea infusions, acid pickled vegetables and even some sausages. The so-called white sandalwood obtained from the trees *Santalum album* (Santalaceae), unlike red sandalwood, is used to flavour foods and other materials, for its high essential oil content (about 2.5%).



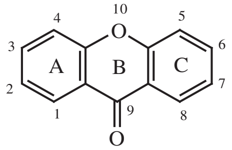
9-108, santalin A, R = OH
santalin B, $R = OCH_3$



9-109, santalin AC

9.5 Xanthones

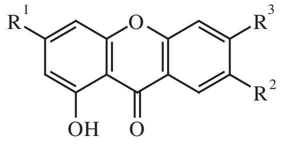
Xanthones are a group of about 70 yellow pigments of basic structure $C_6–C_1–C_6$. They are derived from xanthone (xanthen-9-one, **9-110**) and are biogenetically related to flavonoids. Their occurrence is limited to a few families of higher plants, Clusiaceae (syn. Guttiferae), Gentianaceae, Anacardiaceae, Moraceae and Polygalaceae, some fungi and lichens. From time immemorial, some xanthones have been used as textile dyes and food colorants. Xanthones also exhibit different pharmacological effects and find use, for example, as cardiovascular protectants.



9-110, xanthone

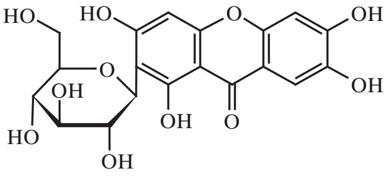
Some important xanthones are the yellow pigments gentisin (1,3,7-trihydroxyxanthone) and gentisein (1,3,6-trihydroxyxanthone, **9-111**) occurring along with other xanthones as glycosides in the yellow gentian root (*Gentiana lutea*, Gentianaceae), which is used for its bitter taste and specific aroma in the production of bitter liqueurs. Gentisein also occurs in centaury (*Centaurium erythraea*), belonging to the same plant family.

A representative of other important xanthones is norathyriol (1,3,6,7-tetrahydroxyxanthone), which occurs as 2-*C*-glucoside



9-111, substituted xanthones

gentisein, R^1 = OH, R^2 = H, R^3 = OH
gentisin, R^1 = OH, R^2 = OH, R^3 = H
norathyriol, R^1 = OH, R^2 = OH, R^3 = OH

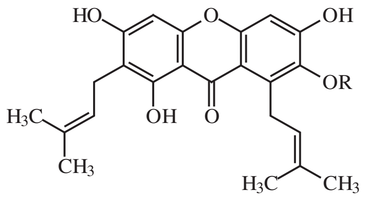


9-112, mangiferin

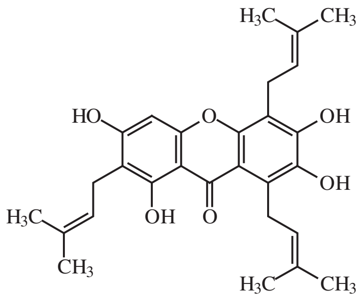
called mangiferin (**9-112**) in a variety of plants and also in the leaves, peels and immature mango fruits. Mangiferin is accompanied by a minor 4-*C*-glucoside known as isomangiferin. The occurrence of mangiferin-6′-*O*-gallate has been described in mango leaves. Mangiferin has anti-inflammatory, antiviral and antioxidant effects. In India, mango tree leaves (*Mangifera indica*, Anacardiaceae) were fed in a limited amount to cattle, whose urine was used as a yellow dye for fabrics and carpets. Biosynthesis of norathyriol starts from phenylalanine and proceeds via cinnamic and benzoic acids, and condensation of this with malonic acid yields 2,4,6-trihydroxybenzofenone. Its oxidation gives 2,3′,4,6-tetrahydroxybenzofenone and its cyclisation provides gentisin and its oxidation norathyriol.

The major secondary metabolites of the tropical tree *Garcinia mangostana* (Clusiaceae) originating in Sunda Islands and the Moluccas, known as mangosteen, are prenylated xanthone derivatives, some of which possess antifungal, antimicrobial, antioxidant and cytotoxic activities. The mangosteen dark purple to red–purple edible fruits have a soft and juicy white aril with a sweet, slightly acid taste and a pleasant aroma. The pericarp of mangosteen fruits has been used in Thai indigenous medicine for the treatment of skin infections, wounds, and diarrhea. The main constituent of the fruit pericarp is yellow xanthone with isoprenoid substituents at positions C-2 and C-8, called α-mangostin, which is accompanied by a number of related pigments, such as γ-mangostin, garcinone E, endraxanthone G, 8-deoxygartanin and gartanin (**9-113**). Another yellow pigment in the mangosteen family is the polyisoprenylated benzophenone derivative garcinol (**9-114**). It occurs at a level of 2–3% in culinary fruits of *G. indica* (and other species), commonly known as kokum. The dried rind of the fruit is used as a garnish for curry and in traditional medicine in India.

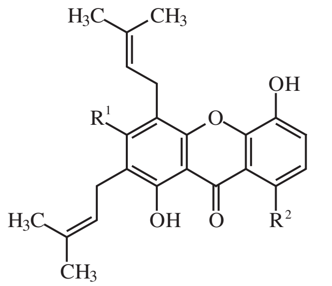
The European mushrooms of the genus *Cortinarius* (Cortinariaceae) characteristically contain the xanthone dermoxanthone (**9-115**) and its methyl ester that were found in the stem of the surprise webcap *C. semisanguineus*. These xanthones are responsible for the bright yellow fluorescence of the mushroom under UV light.



γ-mangostin, R = H
α-mangostin, R = CH_3

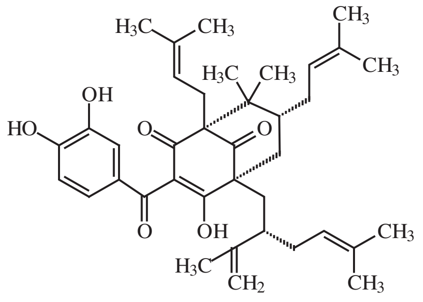


garcinone E

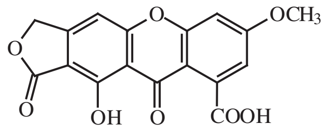


8-deoxygartanin, $R^1 = R^2$ = H
endraxanthone G, $R^1 = CH^3$, R^2 = H
gartanin R^1 = H, R^2 = OH

9-113, xanthones of mangosteen fruits



9-114, garcinol

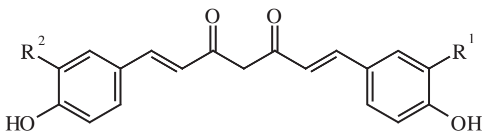


9-115, dermoxanthone

9.6 Curcuminoids

A group of phenolic compounds related to lignans are curcuminoids that are classified as **diarylheptanoids** (C_6–C_7–C_6 compounds). Curcuminoids include the yellow pigments of turmeric (*Curcuma longa*) and other species (about 30 are known) of the genus *Curcuma*. Turmeric, also known as yellow ginger, is a rhizomatous plant from the ginger family (Zingiberaceae), native to India and tropical South Asia. The yellow dried and ground rhizome is used as a spice in curries and to impart colour to various dishes and condiments.

Turmeric contains the diketone curcumin, which is accompanied by demethoxycurcumin and bisdemethoxycurcumin (**9-116**). The predominant pigment is mostly curcumin, (1*E*,6*E*)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-dien-3,5-dione. Its content in the dry rhizome is about 2%; the content of two other pigments is <0.6%. Representation of the pigments may vary according to species, origin and maturity of rhizomes. For example, rhizomes of *C. aromatica* contain only traces of bisdemethoxycurcumin and the main pigment in some species may be demethoxycurcumin.



9-116, turmeric pigments (keto forms)
curcumin, $R^1 = R^2 = OCH_3$
demethoxycurcumin, $R^1 = OCH_3$, $R^2 = H$
bisdemethoxycurcumin, $R^1 = R^2 = H$

Experimental studies demonstrated that curcumin prevents and may even be a cure for Alzheimer's disease and can be a prevention agent and a chemotherapeutic agent for colon cancer. In addition, curcumin shows potent anti-inflammatory and antioxidant activity (the most favoured antioxidant is the enol form). Recombination of curcumin radicals formed in foods yields dimers as radical termination products (Figure 9.19), in addition to the coupling products of curcumin with lipid hydroperoxides.

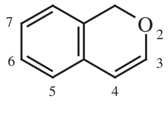
Turmeric is insoluble in water but dissolves well in alcohol and oils. Water-soluble complexes of turmeric are formed by reaction with chlorides of some metals, such as tin and zinc. These complexes have an intense orange colour. The colour of turmeric is virtually stable at typical processing temperatures, but in the light, in the presence of atmospheric oxygen and in alkaline media (the lemon yellow colour at pH = 3 turns to orange–red at pH = 10), turmeric breaks down into colourless products.

Turmeric is mainly used for colouring dairy and bakery products (e.g. ice cream, confectionery and dry mixtures). Oleoresins containing aromatic substances of turmeric are used as a spice. Many species of the genus *Curcuma* (turmeric) and the related genus *Zingiber* (ginger) are locally used as a spice and in traditional medicines.

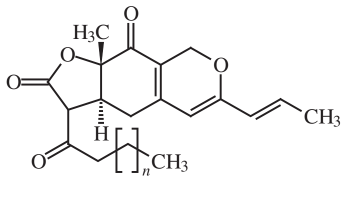
Biosynthesis of curcuminoids is similar to the biosynthesis of phenylpropanoids, such as flavonoids. Intermediates, such as 4-coumaroyl-CoA, condense with malonyl-CoA (via diketide as an intermediate) to form bisdemethoxycurcumin. Curcumin arises by subsequent oxidation and methylation of bisdemethoxycurcumin.

9.7 Isochromenes

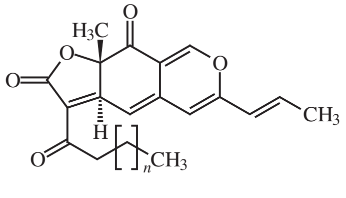
Monascus or monascus red has a certain importance as a food colorant, being a mixture of yellow, orange and red pigments derived from 1*H*-isochromene (**9-117**). In this mixture of lipophilic substances, the yellow pigments monascin and ancaflavin (**9-118**) and their orange oxidised forms rubropunctatin and monascorubin (**9-119**) predominate. These intracellular pigments produce different types of fungi of the genus *Monascus*, especially *M. purpureus*, in solid cultures, for example on cooked rice or bread. Red rice (in China *ankak* or *anka* or *ang khak*, in Japan Beni-Koji or Anka-Koji) dried and crushed to a powder is traditionally used in China, Japan



9-117, 1*H*-isochromene



9-118, monascin, $n = 3$
ancaflavin, $n = 5$



9-119, rubropunctatin, $n = 3$
monascorubin, $n = 5$

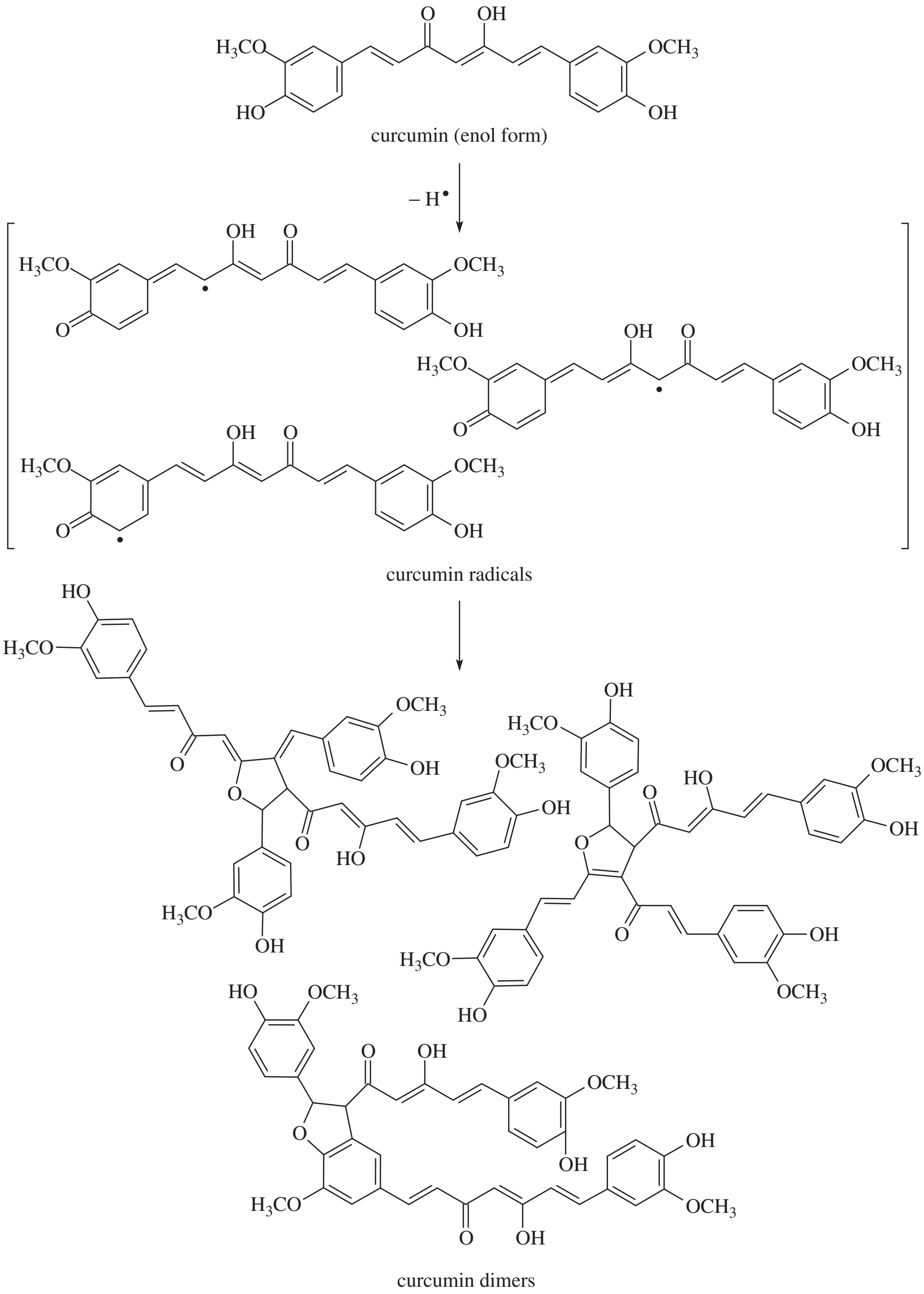
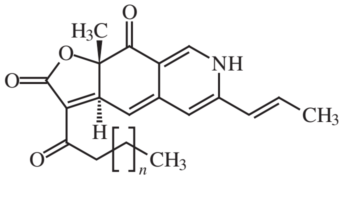


Figure 9.19 Formation of curcumin dimers via radical-radical coupling reaction.

and other countries as a colorant to impart colour to meat, fish, soybean curd (tofu), rice wine (sake), beans, candy and other foods.

Native pigments react easily with amino compounds (amino acids, amino alcohols, amino sugars and proteins) to form extracellular, water-soluble nitrogen analogues of native pigments, which are collectively called **azaphilones**. Red to purple pigments rubropunctamine and monascorubramine (**9-120**) are the nitrogen analogues of yellow pigments, which are formed by reaction with amino compounds.



9-120, rubropunctamine, $n = 3$
monascorubramine, $n = 5$

Many related azafilones produce different Ascomycetes as toxins. Monascus red may even contain mycotoxin citrinin (see Section 12.3.1), which was originally mistakenly regarded as a non-toxic yellow pigment called monascidin A.

The main chromophore of isochromenes arises as a hexaketide from activated forms of acetic and malonic acids and the side chain of pigments is derived from 2-oxohexanic and 2-oxooctanoic acids that are synthesised analogously to other fatty acids.

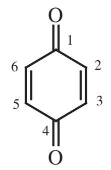
9.8 Quinoid pigments

Plant materials contain a large number of various phenolic substances that give rise to coloured quinones and other pigments formed from phenols by biochemical and chemical reactions. Many of these pigments are also found in foods.

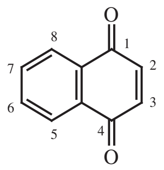
9.8.1 Quinones

Quinones represent a group of about 200 yellow, red, brown and almost black pigments with variable structure. They include simple quinones, dimers, trimers and condensation products that mutually differ in the number of hydroxyl groups and other substituents. The naturally occurring quinoid pigments are mostly derived from:

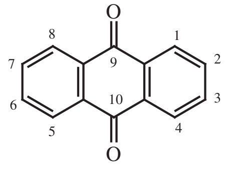
- benzo-1,4-quinone (also known as *p*-benzoquinone or 1,4-dioxobenzene, **9-121**)
- naphtho-1,4-quinone (1,4-dioxonaphthalene, **9-122**)
- anthra-9,10-quinone (9,10-dioxoanthracene, **9-123**).



9-121, benzo-1,4-quinone

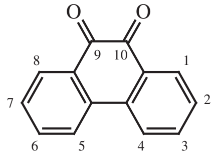


9-122, naphtho-1,4-quinone



9-123, anthra-9,10-quinone

There are also other groups of quinones, common substances are benzo-1,2-quinones (*o*-benzoquinones), which mainly arise in enzymatic browning reactions, and toxic naphtho-1,2-quinone is generated by burning diesel fuel in diesel engines. Related quinones are derived from phenanthra-9,10-quinone (9,10-dioxophenanthrene, **9-124**). Examples of phenanthra-9,10-quinones are furanophenathraquinones occurring in red (Chinese) sage *Salvia miltiorrhiza* root used in traditional medicine (see Section 11.2.2.3.5). Many quinones have more complex structures. For example, a group of terpenoid quinones have been identified that include biologically active plastoquinones, ubiquinones (coenzymes Q), tocopherols and tocotrienols with vitamin E activity, phylloquinone and its derivatives posses vitamin K activity (see Chapter 5).



9-124, phenanthra-9,10-quinone

Quinoid pigments occur in different parts of higher plants, algae, lichens, higher fungi and microorganisms and can also be found in some insects. Quinoid pigments are usually present as differently coloured precursors or in the form of colourless substances, which are mostly reduced forms (leucoforms) of quinones (commonly hydroxy derivatives and their glycosides and esters).

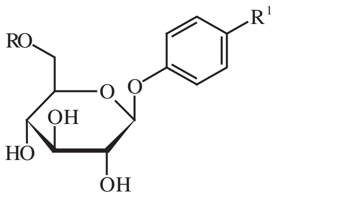
In the past, some quinoid pigments were used as textile and leather dyes (that were later replaced by synthetic dyes) and many plants were specially grown for this function. Some quinoid pigments are now used for cosmetic and pharmaceutical purposes and also as food additives. Compared with other pigments, natural quinoid pigments are of less importance.

9.8.1.1 Benzoquinones

Benzo-1,4-quinoid structures occur in nature as the final oxidation products of various mono- and polycyclic compounds. Most simple benzo-1,4-quinones occur in microorganisms (moulds), higher fungi and lichens, and less frequently in higher plants and some insects. Common substances are glycosides occurring in colourless reduced forms (such as derivatives of hydroquinone known by the systematic name of benzene-1,4-diol). Coloured quinones are formed from these precursors by hydrolysis catalysed by saccharases and by enzymatic oxidation or autoxidation of aglycones.

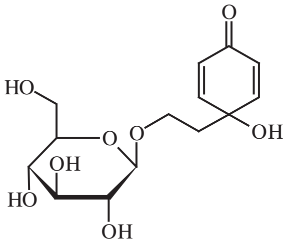
The basic compound is a pale yellow benzo-1,4-quinone, which occurs in plants as a colourless hydroquinone β-D-glucoside called arbutin (**9-125**). This compound is an active component of leaves of the lingonberry (cowberry, *Vaccinium vitis-idaea*) and the related bearberry (*Arctostaphylos uva-ursi*, Ericaceae) native to the northern areas of the Northern Hemisphere. The drug is mainly used as a disinfectant and urinary antiseptic agent. Arbutin is found in the leaves of cowberry in quantities of 3.3 to 5.4% and in bearberry leaves in the amount of 4.2–7.7%. The aglycone hydroquinone is also present at a low level (0.1–0.2% and 0.3%, respectively), which arises via hydrolysis of arbutin by arbutase. Other related glycosides are methylarbutin (1.3%) and pyroside (hydroquinone 6′-acetyl-β-D-glucoside, **9-125**), hydroquinone β-gentiobioside and salidroside (tyrosol β-D-glucoside).

Arbutin is also found in small quantities in cereals (e.g. in wheat and rice) and also in some fruits (especially in pears), and its

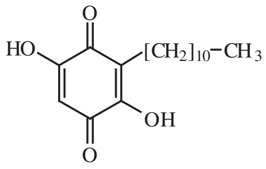


9-125, hydroquinone glucosides
arbutin, R = H, R^1= OH
methylarbutin, R = H, R^1 = OCH_3
pyroside, R = $COCH_3$, R^1 = OH

incidence in fruit juices is an indicator of the presence of pear juice. The arbutin derivative 6′-*O*-(4-hydroxybenzyl)arbutin occurs in marjoram (*Origanum majorana*, Lamiaceae). Also widespread in plants (e.g. in wheat grains) are 2-methoxy and 2,6-dimethoxy derivatives of arbutin, which may cause a pink discoloration in flour. In olives (*Olea europaea*, Oleaceae) a different type of quinoid glycoside occurs, called cornoside (**9-126**). Cornoside is related to oleuropein, which is a bitter substance in olives (see **8-223**). Fruits of *Embelia ribes* (Myrsinaceae), native to India and traditionally used in folk medicine as an anthelmintic drug and to reduce appetite and body weight, and fruits of *Ardisia japonica* of the same plant family, contain yellow–orange 2,5-dihydroxy-3-undecylbenzo-1,4-quinone known as embelin (**9-127**), which is an example of alkyl substituted benzo-1,4-quinones.



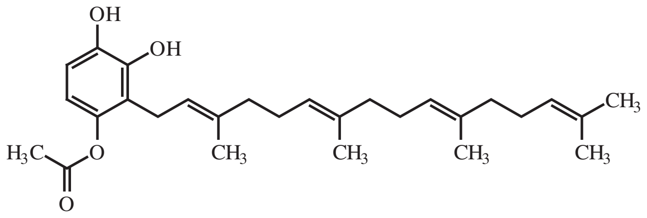
9-126, cornoside



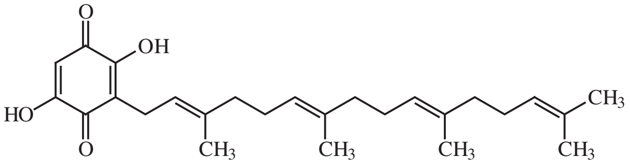
9-127, embelin

Prenylated benzoquinones also occur, albeit rarely, in Boletales mushrooms. Suillin (**9-128**) occurring in weeping (granulated) bolete (*Suillus granulatus*, Suillaceae) is an example of acetylated and prenylated 1,2,4-trihydroxybenzenes. Its oxidation products are responsible for the brown colour of mushroom caps. Prenylated benzoquinones mostly appear as meroterpenoids named boviquinones. Boviquinone-4 (2,5-dihydroxy-3-geranylgeranyl-1,4-benzoquinone, **9-129**) is an example of prenylated benzoquinones found in Jersey cow mushroom (*S. bovines*). The red pigment tridentoquinone (**9-130**) is the main pigment of *S. tridenticus*.

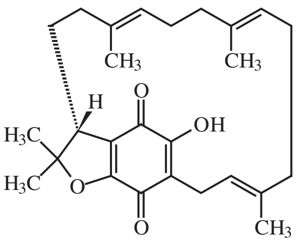
The analogue of agaritine (see Section 10.4) found, for example, in mushrooms of the genus *Agaricus*, which is derived from 4-aminophenol, known as γ-glutamyl-4-hydroxybenzene (**9-131**) readily oxidisese to the corresponding quinone via γ-glutamyl-3,4-dihydroxybenzene. This quinone decomposes to 2-hydroxy-4-iminocyclohexa-2,5-dienone (**9-131**), which imparts a pink–red colour to some agarics (such as common mushroom *A. bisporus*). The yellow nitrogen-containing pigment characteristic of the



9-128, suillin



9-129, boviquinone-4



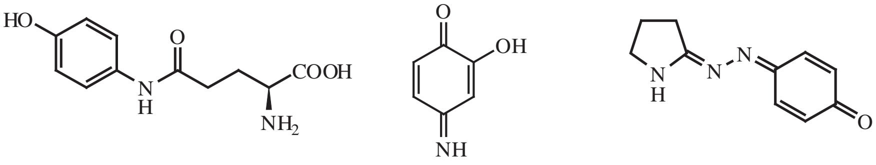
9-130, tridentoquinone

yellow-staining mushroom (*Agaricus xanthodermus*, Agaricaceae) and of some other *Agaricus* species is caused by the metabolite of glutamic acid derived hydrazine agaritine, known as agaricone (**9-131**) that forms by oxidation of the corresponding leucophenol in the damaged tissue.

Benzoquinone carbon atoms can be derived from acetic acid (acetyl-CoA in polyketide quinones) or from glucose (via shikimic acid in microorganisms and fungi). Terpenoid side chains are biosynthesised in the mevalonate (terpenoid) pathway.

9.8.1.1.1 Terphenylquinones

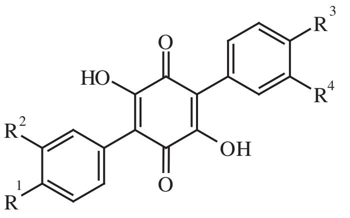
Terphenylquinones (benzo-1,4-quinones substituted at positions C-2 and C-5 by phenyl groups), and their alkyl derivatives are red, violet to brown pigments of many species of lichens, moulds and higher fungi. They often occur in various leuco forms (as dihydroxy derivatives or their acetates). Terphenylquinones, exemplified by the dark red polyporic acid and bronze–brown atromentin (**9-132**), are mainly produced by wood-rotting higher fungi of the order Polyporales growing on various deciduous trees, but in other higher fungi, such as in the Boletales fungi, they appear only sporadically. For example, polyporic acid, the parent compound of numerous terphenylquinones and related compounds, is the major component of *Hapalopilus nidulans* amounting up to 43% of its dry weight. The orange tooth (*Hydnellum aurantiacum*) colour is derived from atromentin and 3,6-dibenzoylatromentin, known as aurantiacin. Atromentin



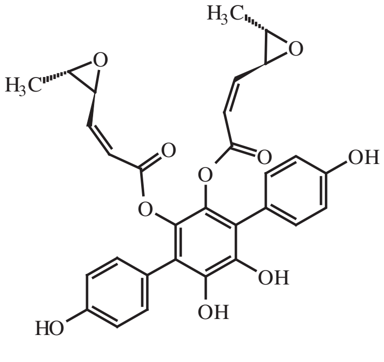
γ-glutamyl-4-hydroxybenzene 2-hydroxy-4-iminocyclohexa-2,5-dienone agaricone

9-131, precursors and pigments in mushrooms of the genus *Agaricus*

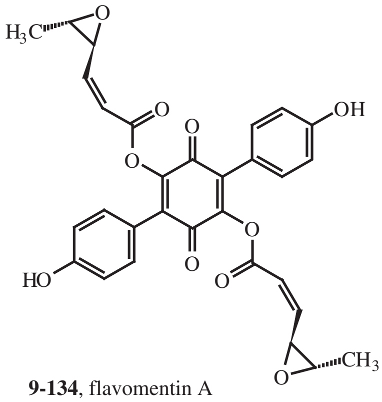
occurs in the intact fruit bodies in the form of colourless precursors, such as dihydroaurantiacin in *Hydnellum aurantiacum* and as leucomentins in a number of other fungi. An example of leuco derivatives of atromentin is leucomentin-2 (**9-133**) esterified with (2*Z*,4*S*,5*S*)-4,5-epoxyhex-2-enoic acid. Atromentin is the key intermediate for many conversions leading to more hydroxylated terphenylquinones (such as variegatin, **9-132**) and other pigments. Mushrooms of the genus *Tapinella* (Tapinellaceae), for example, produce orange–yellow flavomentins and violet spiromentins derived from atromentin. Flavomentins constitute diesters and monoesters of atromentin with (2*Z*,4*S*,5*S*)-4,5-epoxyhex-2-enoic and (2*Z*,4*E*)-hexa-2,4-dienoic acids. Spiromentins possess unique spiro structures in which 4,5-dihydroxybenzo-1,2-quinone is linked to a lactone acetal unit. These metabolites are exemplified by flavomentin A (**9-134**) and spiromentin A (**9-135**).



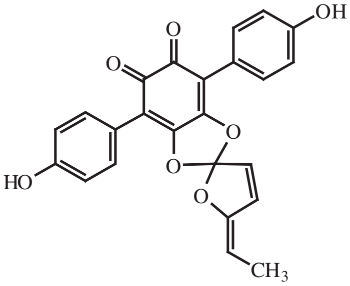
9-132, polyporic acid, $R^1 = R^2 = R^3 = R^4 = H$
atromentin, $R^1 = R^3 = OH$, $R^2 = R^4 = H$
variegatin, $R^1 = R^2 = R^3 = R^4 = OH$



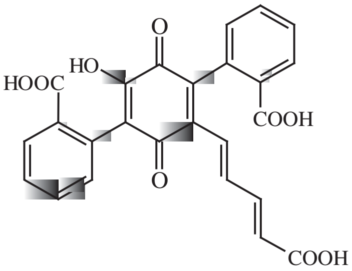
9-133, leucomentin-2



9-134, flavomentin A

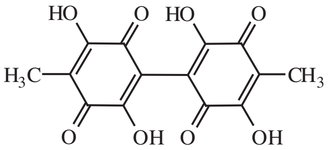


9-135, spiromentin A



9-136, muscarufin

The yellow pigment (2*E*,4*E*)-5-[3,6-dioxo-2,5-di(2-carboxyphenyl)-4-hydroxy-1,4-cyclohexadien-1-yl]-2,4-pentadienoic acid, known as muscarufin (**9-136**), a putative derivative of terphenylquinones, is one of the main pigments of the fly agaric (*Amanita muscaria*) hat. In dimeric forms, terphenylquinones contribute to the yellow colour of some lichens and chocolate brown hats of mushrooms of the order Boletales that are characterised by a diversity of colours. The widespread yellow fungal dimeric metabolite oosporein (**9-137**) shows antifungal, antibiotic and antiviral activities, in addition to being the major secondary metabolite of some fungal biological control agents. For example, it shows growth-inhibitory effect on the phytopathogenic fungus *Phytophthora infestans* (Pythiaceae) that causes a serious disease called late blight in solanaceous crops.

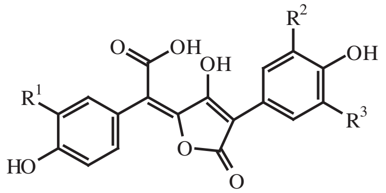


9-137, oosporein

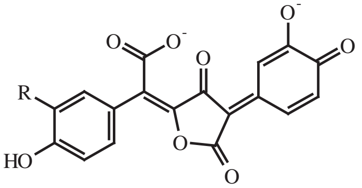
Polyporic acid is biosynthesised by condensation of two molecules of phenylpyruvic acid in the shikimate pathway via the intermediate 2,5-diphenyl-3-hydroxy-4-oxohex-2-enoic acid and by oxidation of the resulting hydroquinone. Terphenylquinones are transformed by enzymatic reactions into pulvinic acids and many other products, some of which are also characteristic pigments of lichens and fungi.

9.8.1.1.2 Pulvinic acids

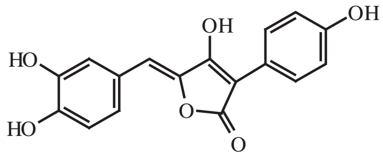
Yellow pigments of lichens, moulds and higher fungi called pulvinic (pulvic) acids (**9-138**) are formed, along with other products, from the corresponding terphenylquinones (**9-132**) through lactone formation, after the terphenylquinone ring has been oxidised and opened. The unsubstituted parent compound, called pulvinic acid, only occurs in the form of its methyl ester, named vulpinic acid. Xerocomic and variegatic acids play the most important role, being responsible for the blue colours acquired in many boletes after their fruiting bodies are injured, which results in the oxidation of these acids to the corresponding blue quinonmethide anions (**9-139**). Pulvinic acids are especially widespread in mushrooms belonging to the genera *Gomphidius* (Gomphidiaceae) and *Suillus* (Suillaceae). The yellow pigment gomphidic acid was found for the first time in the slimy spike-cap (*G. glutinosus*). The yellow–brown cap and stem of the larch bolete (*Suillus grevillei*) contains at least 11 yellow, orange and red pigments derived from decarboxylated pulvinic acids, of which 3′,4′,4-trihydroxypulvinone (**9-140**), derived from variegatic acid (**9-138**), is the major pigment. Simple oxidation products of terphenylquinones under the preservation of the central quinone ring are cycloleucomelone and cyclovariegatin (**9-141**). Cycloleucomelone occurs in the fruiting bodies of *Boletopsis leucomelaena* (Suillaceae) accompanied by a series of colourless analogues containing five, four and three acetyl residues. Cyclovariegatin is a minor pigment of larch bolete and a precursor of violet thelephoric acid (**9-142**). Many other enzymatically catalysed and spontaneous reactions lead to a vast number of other mushroom metabolites. Variegatorubin and xerocomorubin (**9-143**) exemplify red pigments formed from pulvinic acids by the second lactone ring formation. Examples of complex structures derived from pulvinic acids are badione A and norbadione A that are responsible for the chocolate brown and golden yellow colours of the cap skin of bay bolete *Boletus badius* (Boletacaee) (see Section 6.7.4). Oxidative coupling of two molecules of xerocomic acid (**9-138**) yields bright yellow sclerocitrin (**9-144**) found in the fruiting bodies of the common earth ball *Scleroderma citrinum* (Sclerodermataceae), together with norbadione A as the main pigments, which are accompanied by xerocomic acid and badione A. It also occurs as the main pigment of the lemon–yellow coloured stalk base of peppery bolete (*Chalciporus piperatus*, Boletaceae), accompanied by the second main pigment chalcitrin (**9-145**), norbadione A, variegatic acid and variegatorubin.



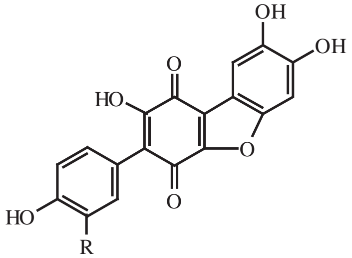
9-138, atromentic acid, $R^1 = R^2 = R^3 = H$
xerocomic acid, $R^1 = H$, $R^2 = OH$, $R^3 = H$
gomphidic acid, $R^1 = H$, $R^2 = R^3 = OH$
isoxerocomic acid, $R^1 = OH$, $R^2 = R^3 = H$
variegatic acid, $R^1 = OH$, $R^2 = OH$, $R^3 = H$



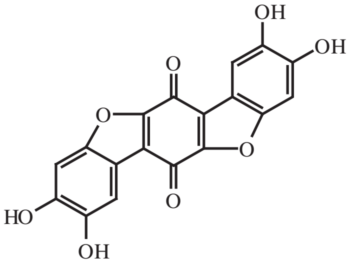
9-139, xerocomic acid qinonemethide anion, R = H
variegatic acid qinonemethide anion, R = OH



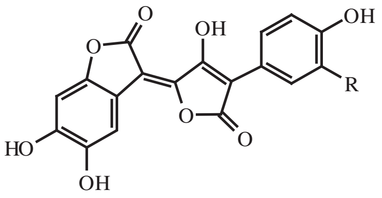
9-140, 3′,4′,4-trihydroxypulvinone



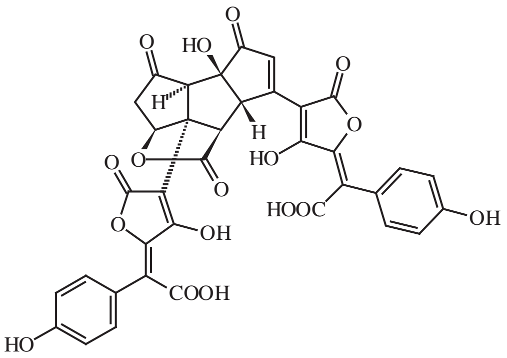
9-141, cycloleucomelone, R = H
cyclovariegatin, R = OH



9-142, thelephoric acid



9-143, xerocomorubin, R = H
variegatorubin, R = OH



9-144, sclerocitrin

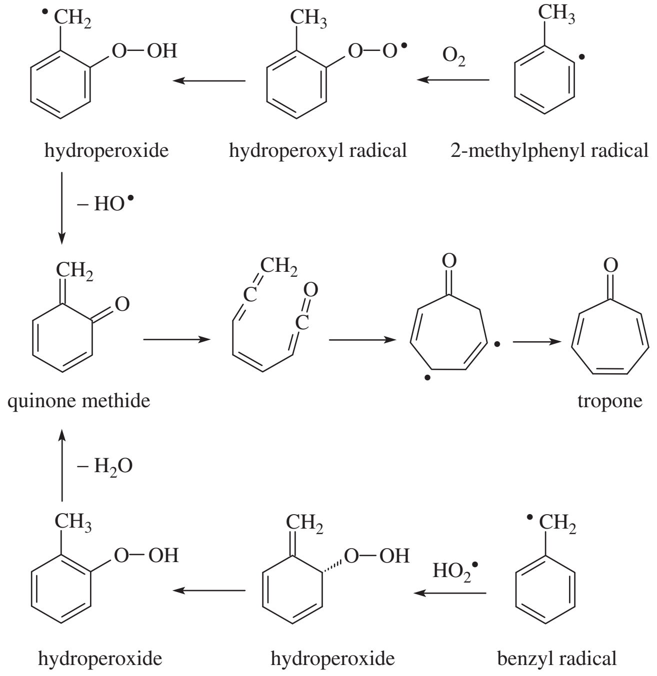
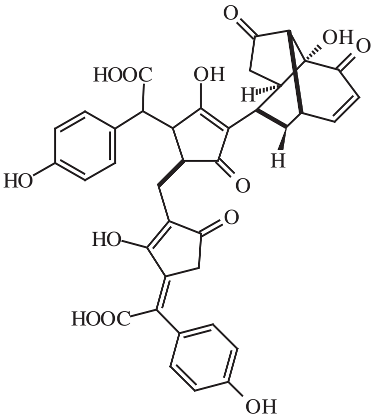


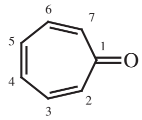
Figure 9.20 Formation of tropone ring from quinone methide.



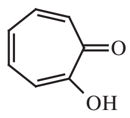
9-145, chalcitrin

9.8.1.1.3 Troponoids

Another group of phenolic compounds are troponoids, compounds derived from tropone (cyclohepta-2,4,6-trien-1-one), which contains as a basic skeleton a seven-membered ring having three conjugated double bonds and a carbonyl group (**9-146**). Troponoids arise by transformation of phenolic compounds and are synthesised mainly by fungi and higher plants, but arise also in non-enzymatic browning reactions. The basic compound with the enol hydroxyl group is 2-hydroxytropone (2-hydroxycyclohepta-2,4,6-trien-1-one), which is called tropolone (**9-147**). Possible pathways leading from quinone methide to tropolone skeleton are outlined in Figure 9.20. Chinone methide arises either from a 2-methylphenyl radical and oxygen or from a benzyl radical and hydroperoxyl (perhydroxyl) radical.

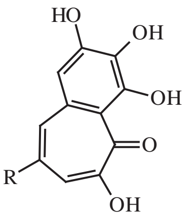


9-146, tropone

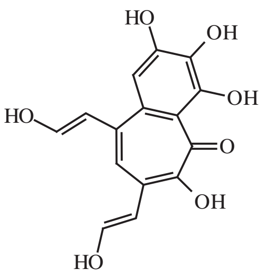


9-147, tropolone

Natural tropone analogues are represented by the brownish-red pigment purpurogallin (**9-148**), occurring, for example, in oak apples, where it arises as a product of gallic acid (gallotannines) oxidation and decarboxylation. Enzymatic oxidation of gallic acid and elimination of carbon dioxide produces its dimer 8-carboxypurpurogallin (**9-148**). A purpurogallin derivative, fomentariol (**9-149**) is produced by the plant pathogen known as the tinder fungus (*Fomes fomentarius*, Polyporaceae). Most troponoids are antibacterial and antifungal agents. Pigments of black tea with a tropone ring (theaflavins and epitheaflavinic acids) are produced from catechins in enzymatic browning reactions (see Section 9.12.4.1).



9-148, purpurogallin, R = H
8-carboxypurpurogallin, R = COOH

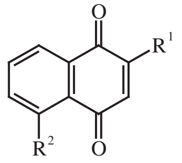


9-149, fomentariol

9.8.1.2 Naphthoquinones

Most naphthoquinones occur in higher plants in the form of glycosides of non-coloured reduced forms. Coloured naphthoquinones are released by glycosidases and enzymatic oxidation or autoxidation of aglycones.

A representative of simple naphthoquinones is yellow–orange lawsone (2-hydroxynaphtho-1,4-quinone, **9-150**), which occurs as a mixture of several naphthoquinones in henna (*Lawsonia inermis*, Lythraceae) growing in the Middle East and India, but also located in many other plants. The pigment, which exhibits antimicrobial effects, is obtained from the leaves of henna bushes, used for centuries for decorating hands, feet, nails and skin, as well as hair dye.

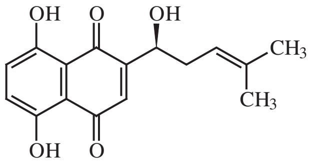


9-150, lawsone, R^1 = OH, R^2 = H
juglone, R^1 = H, R^2 = OH
plumbagin, R^1 = CH_3, R^2 = OH

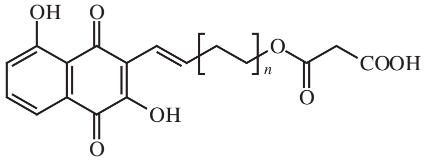
Red–brown juglone (5-hydroxynaphtho-1,4-quinone, **9-150**) is present in the leaves and immature fruits of walnuts (*Juglans regia*, Juglandaceae) and other species as colourless 4-β-D-glucopyranoside of 1,4,5-trihydroxynaphthalene (1,5-dihydroxy-4-naphthalenyl-β-D-glucopyranoside), which is known as hydrojuglone. Juglone arises either by hydrolysis of the glycoside and oxidation of aglycone or by glycoside oxidation and its subsequent hydrolysis. Juglone is an allelopatic substance, which inhibits the growth of many other plants and is responsible for the skin colouring changing to yellow–brown when handling unripe walnuts. Its homologue is a yellow pigment plumbagine (5-hydroxy-2-methylnaphtho-1,4-quinone, **9-150**), which occurs in different parts of the European common leadwort (*Plumbago europaea*, Plumbaginaceae). Plumbagine is also found in the common sundew (*Drosera rotundifolia*, Droseraceae) and other plants. Juglone and plumbagine may produce dermatitis in susceptible people, and juglone has a laxative effect in the same way as some anthraquinones.

The roots of alkanet, also known as common bugloss (*Alkanna tinctoria*, syn. *Anchusa tinctoria*, Boraginaceae) growing in the south of Europe, contain the reddish pigment alkannin (alkannet, **9-151**) that was used as a textile dye. The (*S*)-isomer of alkannin is used today as a food colouring E103 to impart colour to ice creams, sweets and other products. The red (*R*)-isomer called shikonin was isolated from the roots of the medicinal plant *Lithospermum erythrorhizon* of the same family, which is native to China.

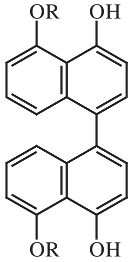
Naphthoquinone derivatives are also common secondary metabolites of slime molds of the class Myxogastria (formely known as Myxomycota). Examples are the red pigments trichione and homotrichione (**9-152**) of the sporophores of *Trichia floriformis*. Naphthalene and naphthoquinone structures are also widespread as red, purple, brown and black pigments in higher



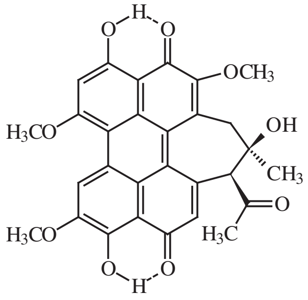
9-151, alkannin



9-152, trichione, *n* = 1
homotrichione, *n* = 2



9-153, 1,1′,8,8′-tetrahydroxy-4,4′-binaphthyl, R = H
1,8-dihydroxy-1′,8′-dimethoxy-4,4′-binaphthyl, R = CH_3



9-154, hypocrellin

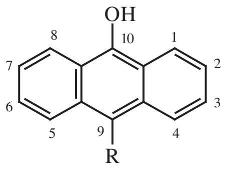
fungi of the phylum Ascomycota. For example, xylariaceous wood fungi of the genus *Daldinia* (Xylariaceae) are dominated by metabolites produced by oxidative coupling of colourless naphthalene-1,8-diol. Examples of these structures are 4,4′-binaphthyl derivatives 1,1′,8,8′-tetrahydroxybinaphthyl and its ether 1,8-dihydroxy-1′,8′-dimethoxybinaphthyl (**9-153**) and more condensed brown-to-black pigments called perylenequinones. A simpler perilenequinone known as hypocrellin (**9-154**), isolated from the bamboo fungus *Hypocrella bambusae* (Hypocreaceae), is a photodynamic pigment.

Biosynthesis of naphthoquinones lawsone and juglone starts, similarly to phylloquinone biosynthesis, from the shikimic acid metabolite chorismic acid, and proceeds via the intermediate 1,4-dihydroxy-2-naphthoic acid. The precursor of alkannin is 4-hydroxybenzoic acid, which is another metabolite of

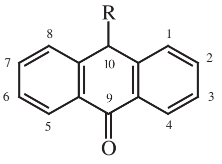
shikimic acid. Isoprenoid substituents are derived from geranyl diphosphate in the mevalonate pathway. Naphthoquinone plumbagin and naphthoquinones of fungi are examples of naphthoquinones arising exclusively by the acetate–polymalonate pathway via corresponding ketides.

9.8.1.3 Anthraquinones

Anthraquinones are the most widespread group of natural quinones. They occur in higher plants, fungi, lichens and some insects. Anthraquinones are accompanied by derivatives of anthranol, anthrahydroquinone (**9-155**) and their oxo forms anthrone and oxanthrone (**9-156**), which are, as glycosides, precursors of anthraquinone pigments.

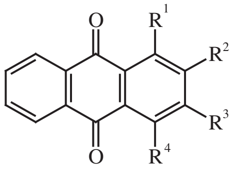


9-155, anthranol, R = H
anthrahydroquinone, R = OH



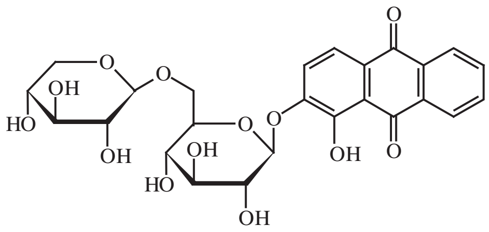
9-156, anthrone, R = H
oxanthrone, R = OH

The most familiar single anthraquinone substituted with hydroxyl groups on only one benzene ring is yellow–orange 1,2-dihydroxyanthraquinone, called alizarin (or Turkish Red or Mordant Red 11), and red pigment 1,2,4-trihydroxyalizarin, known as purpurin (**9-157**) that occura in roots of the common madder (*Rubia tinctorum*, Rubiaceae). Other anthraquinone aglycones in madder are lucidin and quinizarin (**9-157**). Alizarin occurs as a glycoside known as ruberythric acid (**9-158**), the sugar moiety of which is primeverose (6-*O*-β-D-xylopyranosyl-β-D-glucopyranose) bound to the C-2 alizarin hydroxyl group. Another main glycoside is lucidin-3-*O*-primeveroside. The pigments arise from glycosides by fermentation or acid hydrolysis. Related anthraquinone anthragallol (**9-157**) occurs with its hydroxy derivatives, methyl ethers, glycosides and other derivatives in a number of plants.

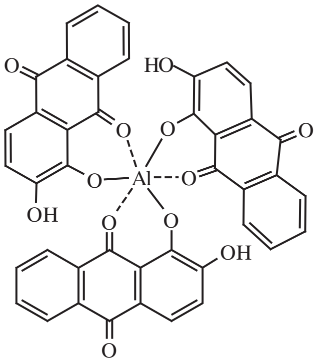


9-157, alizarin, R^1 = OH, R^2 = OH, R^3 = H, R^4 = H
purpurin, R^1 = OH, R^2 = OH, R^3 = H, R^4 =OH
lucidin, R^1 = OH, R^2 = CH_2OH, R^3 = OH, R^4 = H
quinizarin, R^1 = OH, R^2 = H, R^3 = H, R^4 = OH
anthragallol, R^1 = OH, R^2 = OH, R^3 = OH, R^4 = OH

Alizarin is used as a dye mostly for textile purposes, but also for food colouring. Purpurin is normally not coloured, but is red when dissolved in alkaline solutions. Mixed with clay and treated with alum and ammonia, it gives a brilliant red colorant madder lake (**9-159**).



9-158, ruberythric acid

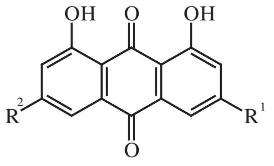


9-159, madder lake

Anthraquinone pigments in madder are biosynthesised by the shikimate (chorismate) pathway, which provides only anthra-9,10-quinones. The starting compound is naphtho-1,4-quinone-2-carboxylic acid.

9.8.1.3.1 Emodins

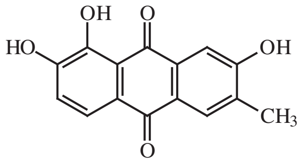
A very important group of polyhydroxysubstituted anthraquinones substituted at both aromatic rings are emodins (**9-160**). A common feature of these anthraquinones is the presence of at least two OH groups (at C-1 and C-8) and a methyl group (at C-3) or its oxidised forms (hydroxymethyl or carboxyl group).



9-160, chrysophanol, R^1 = CH_3, R^2 = H
emodin, R^1 = CH_3, R^2 = OH
aloe-emodin, R^1 = CH_2OH, R^2 = H
rhein, R^1 = COOH, R^2 = H
parietin, R^1 = CH_3, R^2 = OCH_3

Emodin (formerly also known as frangula-emodin) is widespread in fungi, lichens and higher plants, some of which are used in medicine. It occurs, for example, in the bark and fruits of common shrubs of the genus *Frangula* (such as alder buckthorn, *F. alnus*) and *Rhamnus* (such as buckthorn, *R. cathartica*) of the Rhamnaceae family and in the leaves and pods of Egyptian senna (*Senna alexandrina*, syn. *Cassia angustifolia*, Fabaceae). The aqueous extract of

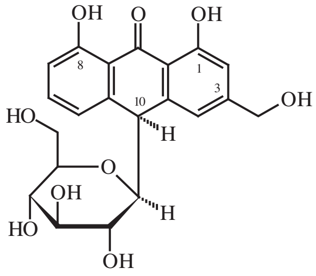
Egyptian senna is allowed as a dye for cigarette paper. Chrysophanol, emodin, rhein and parietin (also known as physcion) and chrysarone (**9-161**) are found in the red crisp stalks and especially in the roots of various species of rhubarbs (*Rheum* spp., Polygonaceae). Aloe-emodin is found in some aloe species (*Aloe* spp., Xanthorrhoeaceae) and is also synthesised by the mold *Aspergillus wentti*, which is used for the production of pectolytic enzymes. Parietin occurs in lichens of the genus *Xanthoria*.



9-161, chrysarone

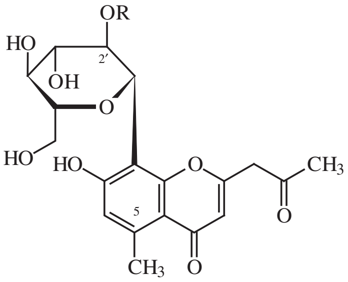
Like other quinones, emodins occur in plants as glycosides. For example, in rhubarb (*R. rhabarbarum*, syn. *R. undulatum*, and false rhubarb *R. rhaponticum*), chrysophanol is present as 1-*O*-β-ᴅ-glucopyranoside, called chrysophanein or chrysophaniin, and parietin occurs as 1-*O*-glucoside, known as physcionin. The same glycosides and chrysophanol-8-*O*-glucoside, called pulmatin, occur in *R. pulmatum*. In young leaves glycosides derived from anthranols are prevalent, while in older leaves anthraquinone glycosides prevail.

Plants of the genus *Aloe* contain a reduced form of aloe-emodin as the *C*-glucoside of the corresponding anthrone, which is called aloin. Lemon yellow aloin is a mixture of two isomers. The major product (*S*)-aloin or (*S*)-β-ᴅ-glucopyranoside-aloe-emodin is referred to as aloin A (or barbaloin, **9-162**), the (*R*)-isomer is aloin B (isobarbaloin). Related glycosides called aloinosides *a* and *b* contain α-ʟ-rhamnopyranose bound by an *O*-glycosidic bond to the hydroxymethyl group at C-3.



9-162, aloin A

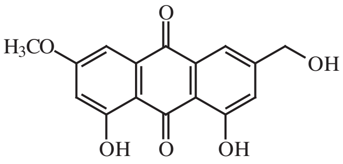
Some emodins have a laxative effect and relevant purgative drugs (containing about 10% of aloin) were used for centuries in medicine. Aloin also shows retching and mutagenic effects. *Aloe* spp. are largely used in foods and beverages as flavoring agents. Because of the averse pharmacological effects of aloe constituents on consumers, the EEC listed aloin as a marker of *Aloe* occurrence in food and limited the amount of aloin to levels of 0.1 mg/kg in foods, 0.1 mg/l in beverages and 50 mg/l in alcoholic beverages. The choice of aloin as a marker of aloe in beverages is inadequate since aloin is unstable in aqueous and alcoholic solutions. The degradation products include aloin dimers and trimers and aloe-emodin, therefore more stable 5-methylchromones aloesin and aloeresin A (**9-163**) were suggested as alternative markers for the presence of aloe in alcoholic beverages.



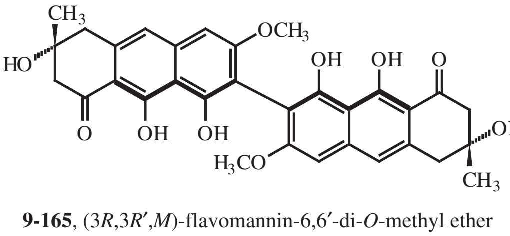
aloesin, R = H
aloeresin A, R = *p*-coumaroyl

9-163, 5-methylchromones of *Aloe* spp.

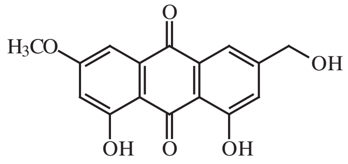
Fungi contain a range of anthraquinones of octaketide origin with both rings substituted. In many cases, anthraquinones are found in fungi as the corresponding colourless reduced forms that may occur as glycosides. Many natural anthraquinones are oligomers formed by the coupling of two or more anthraquinone molecules. These oligomers further differ in the points through which monomers are attached and may have more than one polymorphic form. For example, the major dark orange pigment of the European toadstool *Cortinarius cinnabarinus* (Cortinariaceae) is fallacinol (6-*O*-methoxycitreorosein, **9-164**). Several species of the genera *Cortinarius*, *Dermocybe* and *Tricholoma*, such as *C. cinnamomeoluteus*, trivially known as man on horseback, produce a bright yellow dimeric anthraquinone flavomannin-6,6′-di-*O*-methyl ether (**9-165**). This pigment is biosynthesised by 7,7′-coupling of the corresponding green dihydroanthracenone (*R*)-torosachrysone (**9-166**). In its homochiral form it also occurs in the European *C. citrinus* and *C. croceus*, while in Australian fungi it forms a mixture of (3*R*,3*R*′,*M*)- (**9-165**) and (3*R*,3*R*′,*P*)-atropoisomers. The green (*R*)-atrochrysone (**9-166**) occurs in *C. atrovirens* and *C. odoratus*, (*S*)-torosachrysone-8-*O*-methyl ether is a constituent of *C. fulmineus*, *C. citrinus*, *C. splendens*, *T. equestre* and the sulfur knight *T. sulfureum*.



9-164, fallacinol



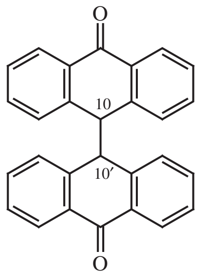
9-165, (3*R*,3*R*′,*M*)-flavomannin-6,6′-di-*O*-methyl ether



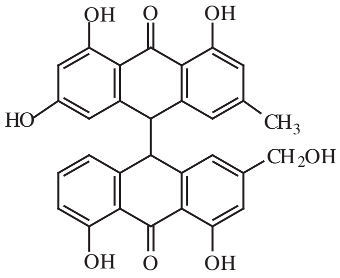
9-166, (*R*)-atrochrysone, R = H
(*R*)-torosachrysone, R = CH_3

9.8.1.3.2 Bianthrones and related compounds

In addition to emodins, rhubarb root also contains dimeric reduced forms of emodins derived from 10,10′-bianthronyl (**9-167**), which are called bianthrones (dianthraquinones). They can occur as homobianthrones, such as emodin bianthrone and parietin bianthrone, or as heterobianthrones (mixed dimers), such as palmidin A (**9-168**), which is bianthrone of emodin and aloe-emodin.



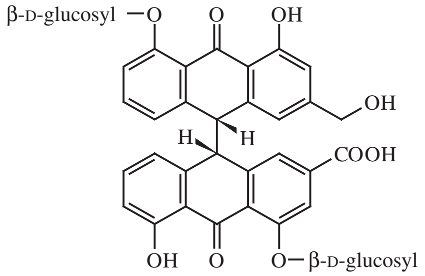
9-167, 10,10′-bianthronyl



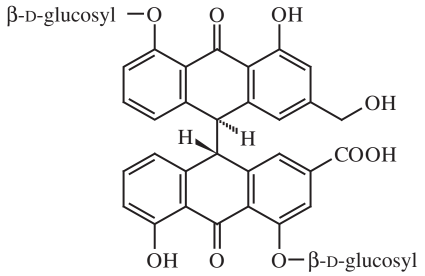
9-168, palmidin A

The related sennosides, glycosides A, B, C and D, occur in amounts of 1.5–3% in the leaves of Egyptian senna shrubs that are cultivated primarily in India. Senna leaves were previously used by Arab physicians as a laxative drug and digestive stimulant. Heterobianthrones sennosides A (*meso*-derivative, **9-169**) and B (*trans*-derivative, **9-170**) are bianthrone glycosides derived from aloe-emodin and rhein, homobianthrones sennosides C (*meso*-derivative) and D (*trans*-derivative) are derived only from rhein.

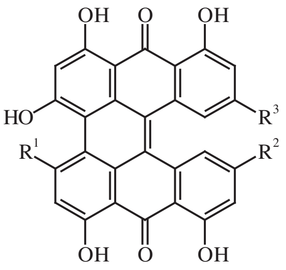
An even more condensed derivative of emodin bianthrone is the purple photodynamic pigment hypericin, 1,3,4,6,8,13-hexahydroxy-10,11-dimethylphenanthro[1,10,9,8-*opqra*]perylen-7,14-dione (**9-171**). Hypericin is a pigment of the flowers of St. John's wort (*Hypericum perforatum*, Hypericaceae), a yellow-flowering perennial herb indigenous to Europe, and other



9-169, sennoside A



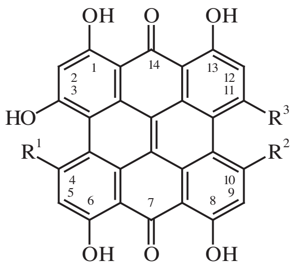
9-170, sennoside B



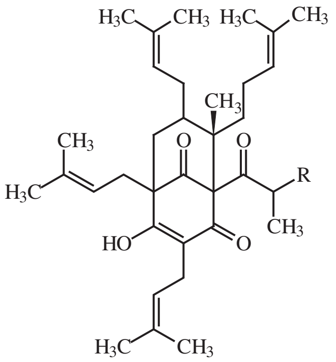
9-171, protohypericin, $R^1 = OH$, $R^2 = CH_3$, $R^3 = CH_3$
protopseudohypericin, $R^1 = OH$, $R^2 = CH_3$, $R^3 = CH_2OH$

Hypericum species, which is accompanied by related pigments pseudohypericin and isohypericin and (**9-172**) and their precursors protohypericin and protopseudohypericin, phloroglucinols (hyperforin and adhyperforin, **9-173**), flavonol glycosides and biflavones. Hyperflorin and its derivative adhyperflorin inhibit different receptor neurotransmissions (act as inhibitors of hormones catecholamines, such as serotonin, and of GABA and glutamate) and have antidepressant effects for which the plant is widely known as a herbal treatment for depression. A pigment structurally related to hypericin is the dark red pigment phagopyrin (**9-174**), 1,3,4,6,8,13-hexahydroxy-10,11-dimethyl-2,5-di-2-piperidinylphenanthro[1,10,9,8-*opqra*]perylen-7,14-dione that is found mainly in flowers and herbs of common buckwheat (*Fagopyrum esculentum*) of the family Polygonaceae. Hydrolysis of phagopyrin provides hypericin and other products. Consumption of phagopyrin and hypericin and subsequent irradiation by sunlight leads to phagopyrism, which was observed in white and white-spotted animals (sheep, pigs, cattle and horses) that were fed buckwheat leaves. The presence of hypericin in food is consequently regulated by legislation. According to Regulation (EC) No. 1334/2008,

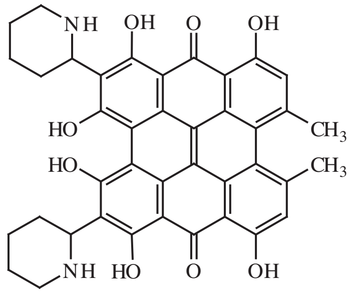
flavourings and food ingredients with flavouring properties produced from *Hypericum perforatum* may only be used for the production of alcoholic beverages.



9-172, hypericin, R^1 = OH, R^2 = CH_3, R^3 = CH_3
pseudohypericin, R^1 = OH, R^2 = CH_3, R^3 = CH_2OH
isohypericin, R^1 = CH_3, R^2 = OH, R^3 = CH_3



9-173, hyperflorin, R = CH_3
adhyperflorin, R = CH_2CH_3

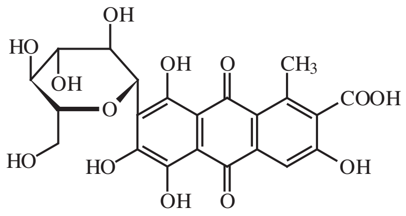


9-174, phagopyrin

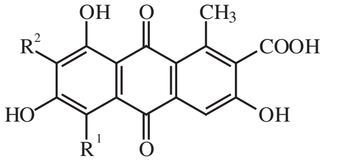
Emodins with both aromatic rings hydroxylated are synthesised exclusively by the polyketide pathway as octaketides. Biosynthesis of bianthrones and other condensed emodin derivatives occurs by one-electron oxidation of anthrone derivatives (anthranols) to radicals, which are joined to form bianthrones.

9.8.1.3.3 Carmine pigments

Carmine pigments are anthraquinone derivatives, which, unlike other anthraquinones, are of animal origin. **Carmine** (also known as Natural Red 4) is the term that describes the aluminium chelate of anthraquinone derivative carminic acid. The term **cochineal** is used to describe both the fertilised females of the coccid insect *Dactylopius coccus* (*Coccus cacti*, Dactilopiidae), from which carmine is derived, and also the pigments obtained. The insects live as a parasites on cacti of genera *Opuntia* and *Nopalea* (Cactaceae) and especially on *N. coccinellifera*, which was originally grown in Mexico. Dry and milled insect bodies are used as the pigment, and the pigment can constitute up to 22% of solids. The main component of cochineal is carminic acid (**9-175**), which is present as *C*-glucoside, 7-α-D-glucopyranosyl-9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracenecarboxylic acid, the aglycone of which is the anthraquinone derivative 9,10-dihydro-3,5,6,8-tetrahydroxy-1-methyl-9,10-dioxo-2-anthracenecarboxylic acid, which is called kermesic acid (**9-176**).



9-175, carminic acid

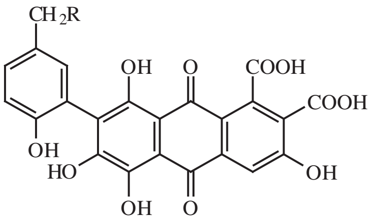


9-176, kermesic acid, R^1 = OH, R^2 = H
ceroalbolinic acid, R^1 = H, R^2 = OH

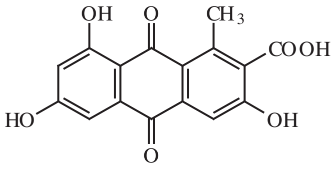
Carminic acid is a water-soluble and stable substance. The shade of colour in solution depends on pH and is orange at pH 3, red at pH 5.5 and purple at pH 7. The colour intensity is relatively low, therefore carminic acid is used mainly as the red aluminium salt called carmine lake or crimson lake (containing about 50% of carminic acid), which is prepared from powdered insect bodies hydrolysed in diluted ammonia (or sodium carbonate) and by addition of alum. It is used as an intense red pigment for colouring some aperitifs (vermouths), meat products, specialty bakery and confectionery products, jams and dairy products.

A similar pigment to carmine is **kermes** that was mainly used in the Middle Ages in the Mediterranean region. Kermes is derived from European species of insects, mainly of *Kermes vermilio and K. illicis* (Kermesidae) living on the kermes oak (*Quercus coccifera*, Fabaceae). The main pigment of kermes is kermesic acid, the same aglycone as in carminic acid. Along with kermesic acid, isomeric ceroalbolinic acid (**9-176**) also occurs.

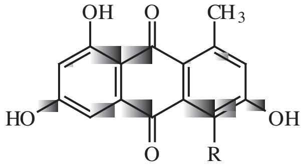
Lac is the scarlet secretion of a number of species of insects of several genera, of which the most commonly cultivated species is *Kerria lacca* (syn. *Laccifer lacca*, Kerriidae). The main producers of lac are India and Malaysia. Lac pigment is a complex mixture of laccaic acids (**9-177** and **9-178**), erythrolaccin and deoxyerythrolaccin (**9-179**).



9-177, laccaic acid A, R = $CH_2NHCOCH_3$
laccaic acid B, R = CH_2OH
laccaic acid C, R = $CH(NH_2)COOH$
laccaic acid E, R = CH_2NH_2



9-178, laccaic acid D



9-179, erythrolaccin, R = OH
deoxyerythrolaccin, R = H

9.9 Carotenoids

Carotenoids are widespread yellow and orange (rarely yellow–green and red) lipophilic pigments of plants, fungi, algae, microorganisms and some animals (crustaceans, fish, birds and mammals). Their annual production in nature is estimated at 1×10^8 tons. In plants, carotenoids are principally associated with chlorophylls in plastids called chromoplasts and chloroplasts. Today, about 700 naturally occurring carotenoid pigments are known. Of this number, about 50 compounds may act as provitamins A.

9.9.1 Structure and nomenclature

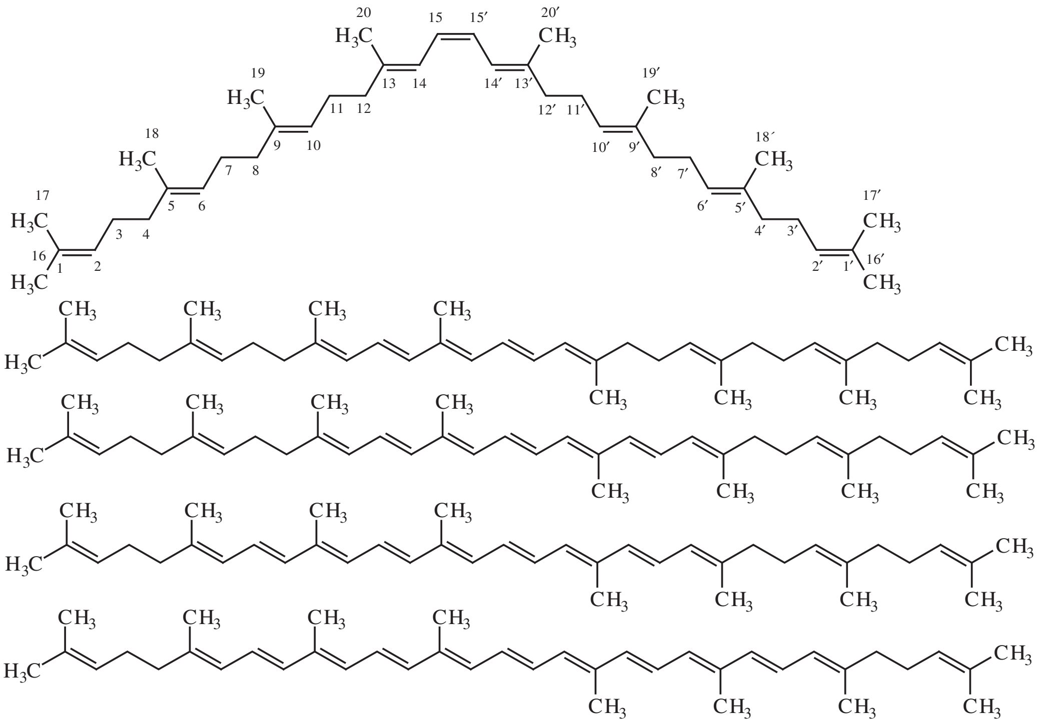
Most carotenoid compounds are tetraterpenoids formally containing eight isoprene units. They owe their colour to the chain of conjugated double bonds, which occurs in several basic structures and their combinations.

Carotenoids are divided into two main groups:

- hydrocarbons called **carotenes**
- oxygen-containing compounds (alcohols, aldehydes, ketones, epoxides and other compounds) derived from carotenes, which are known as **xanthophylls**.

9.9.1.1 Carotenes

The simplest prototype of carotenes, called **ψ-carotenes**, is the polyunsaturated acyclic hydrocarbon (15*Z*)-7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene, known as phytoene, which is synthesised from two molecules of geranylgeranyl diphosphate. Isomerisation of phytoene yields the *trans*-isomer phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene). Oxidation of phytofluene gradually gives ζ-carotene (7,8,7′,8′-tetrahydro-ψ,ψ-carotene), neurosporene (7,8-dihydro-ψ,ψ-carotene) and lycopene(ψ,ψ-carotene), which is the final product of the biosynthesis (**9-180**) and the main pigment of tomatoes (30–200 mg/kg), watermelons (33–121 mg/kg) and rose hips (101–834 mg/kg



9-180, acyclic carotenes

fresh weight). Acyclic carotenes, with the exception of lycopene, are found in food materials in small quantities. They accompany alicyclic carotenes and xanthophylls, which are the main carotenoids.

Alicyclic carotenes are formed by enzymatically catalysed cyclisation at one or both ends of the acyclic ψ-carotenes, which leads to formation of a β-ionone structure in **β-carotene**s or α-ionone structure in **ε-carotene**s (Figure 9.21). Examples of hydrocarbons with a β-ionone ring at only one end of the molecule are β-zeacarotene and γ-carotene (β,ψ-carotene). Cyclisation at both ends of the molecule produces structures that are present, for example, in β-carotene, α-carotene or α-zeacarotene. β-Carotene with two β-ionone rings is therefore systematically called β,β-carotene, α-carotene is β,ε-carotene, because it has only one β-ionone ring and one α-ionone ring. Carotene with two α-ionone rings (ε-rings) is ε-carotene or ε,ε-carotene. Carotenes with a β-ionone ring, such as α-carotene, β-carotene and γ-carotene, are precursors of retinol and are, therefore, provitamins A. β-Zeacarotene is not provitamin A, because it has a partially reduced side chain (**9-181**).

9.9.1.2 Xanthophylls

Xanthophylls are the main carotenoids of plants. They primarily arise as products of biochemical oxidation (hydroxylation and epoxidation) of carotenes. Xanthophylls derived from acyclic carotenes occur in foods in small quantities. For example, tomatoes contain as minor pigments 1,2-epoxylycopene, 5,6-epoxylycopene, 1,2-epoxyphytoene and some other compounds. Much more common are monohydroxysubstituted alicyclic derivatives of carotenes called **cryptoxanthins**. Most plant materials contain small amounts of α-cryptoxanthin also called zeinoxanthin, derived from α-carotene (**9-182**) and β-cryptoxanthin, derived from

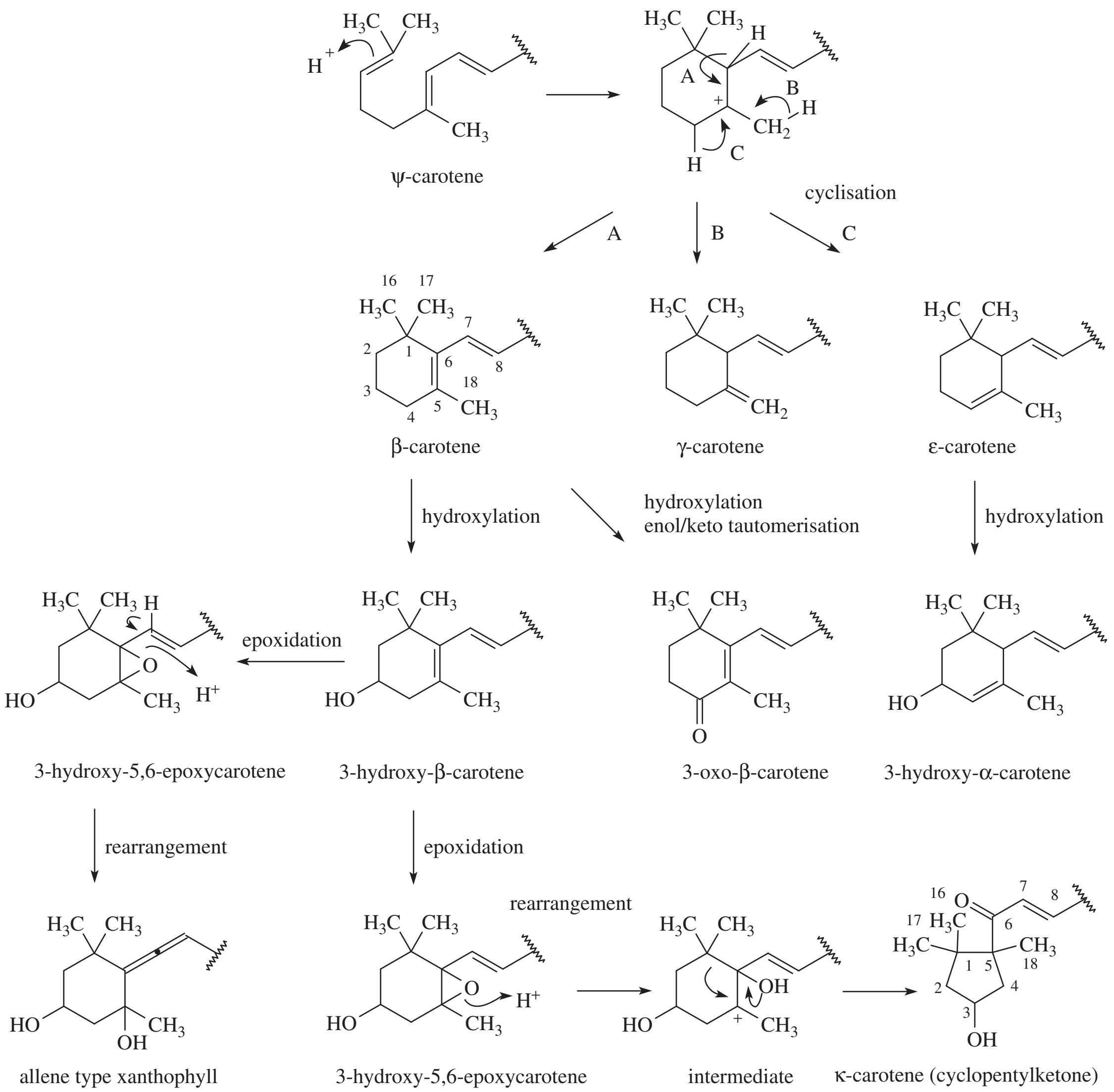
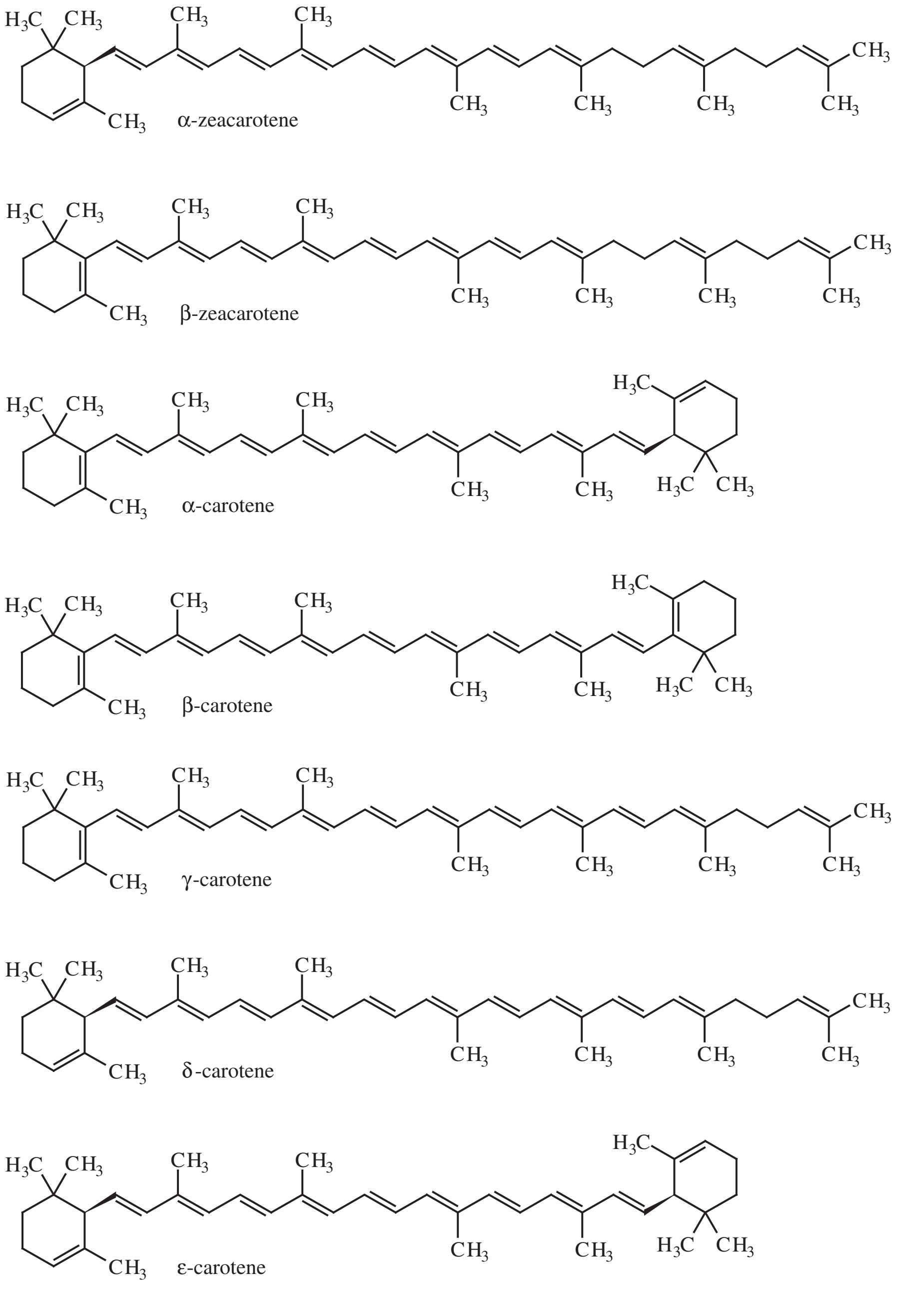


Figure 9.21 Formation of alicyclic carotenes and xanthofylls.

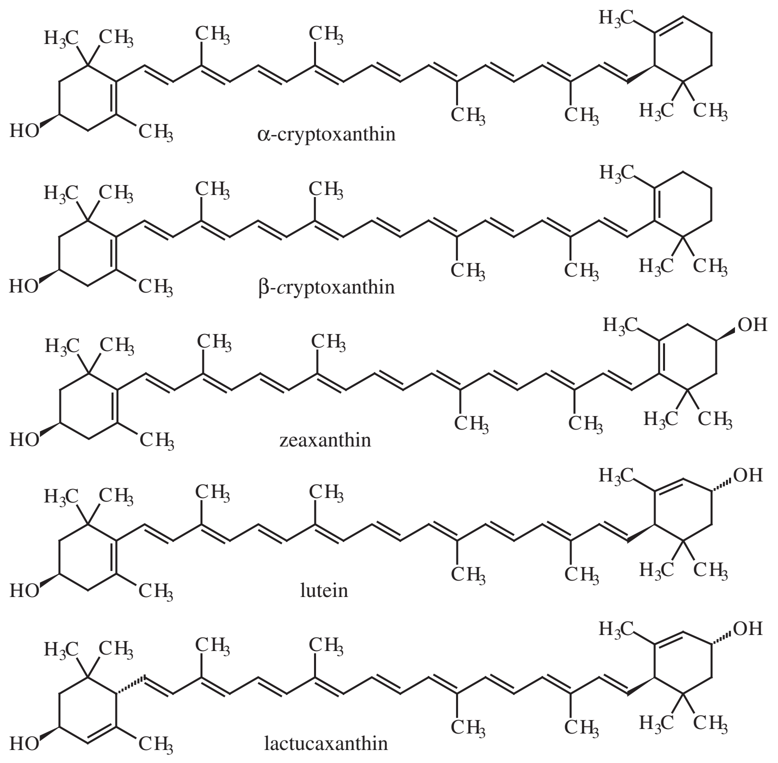


9-181, alicyclic carotenes

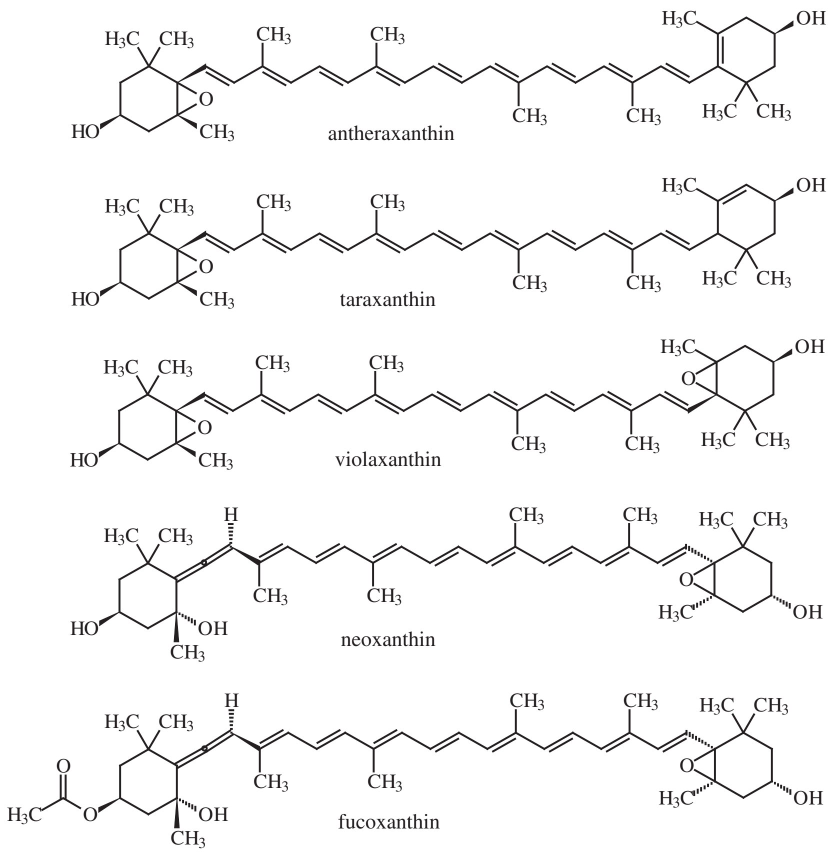
β-carotene, which are precursors of xanthophylls containing two hydroxyl groups in the molecule. The xanthophyll β-cryptoxanthin is provitamin A. Examples of dihydroxysubstituted pigments are zeaxanthin (arising from β-cryptoxanthin and β-carotene, respectively) and lutein (the precursor of which is α-cryptoxanthin and α-carotene, respectively), the precursor of lactucaxanthin is ε-carotene.[4]

Oxidation of these compounds subsequently gives rise to 5,6-epoxides, such as antheraxanthin (**9-183**) derived from β-carotene or taraxanthin (lutein epoxide) derived from α-carotene. Oxidation

[4]The systematic name of zeaxanthin is (3*R*,3′*R*)-7,8-didehydro-β,β-carotene-3,3′-diol or 3,3′-dihydroxy-β-carotene, lutein is (3*R*,3′*S*,6′*R*)-β,ε-carotene-3,3′-diol or 3,3′-α-carotene. Antheraxanthin is 5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol, taraxanthin is (3*S*,5*R*,6*S*,3′*S*,6′*R*)-5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3′-diol, violaxanthin is (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol or zeaxanthin diepoxide. Neoxanthin is (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-5′,6′-diepoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol. Mutatochrom (also known as citroxanthin) is 5,8-epoxy-5,8-dihydro-β,β-carotene, mutatoxanthin is 5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol, luteoxanthin is 5,6,5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β,β-carotene-3,3′-diol, auroxanthin is 5,8,5′,8′-diepoxy-5,8,5′,8′-tetrahydro-β,β-carotene-3,3′-diol. Capsanthin is (3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β,κ-caroten-6′-one, capsorubin is (3*S*,5*R*,3′*S*,5′*R*)-3,3′-dihydroxy-κ,κ-caroten-6,6′-dione, cryptocapsin is (3′*S*,5′*R*)-3′-hydroxy-β,κ-caroten-6′-one.



9-182, hydroxy and dihydroxy carotenes



9-183, 5,6-epoxides and allenes

at both ends of the molecule gives 5,6,5′,6′-diepoxides, such as violaxanthin. Neoxanthin, formerly also called foliaxanthin, which is found in higher plants, and fucoxanthin, occurring in algae, are examples of rarely occurring compounds called **allenes** (dienes with cumulated double bonds).

Isomerisation of 5,6-epoxides yields both isomers of the corresponding 5,8-epoxides. Examples are mutatochrom (derived from β-cryptoxanthin, **9-184**) and mutatoxanthin (derived from zeaxanthin). Luteoxanthin and auroxanthin are both 5,6- and 5,8-diepoxides. By rearrangement of the 5,6-epoxy group, an additional group of xanthophylls arises that are also called cyclopentylketones or κ-carotenes (**9-185**). The most important κ-carotenes are capsanthin and capsorubin. Smaller amounts of cryptocapsin, capsanthon, capsochrom and capsanthin epoxides also can be found. The reaction mechanisms of biosynthesis of most xanthophylls are shown schematically in Figure 9.21.

9.9.1.3 Apocarotenoids

A relatively small, but very important group of xanthophylls are compounds containing less than 40 carbon atoms in the molecule. These compounds, resulting from the breakdown of carotenoids, are called **degraded carotenoids** or **apocarotenoids**. Apocarotenoids exhibit different biological functions. The most important apocarotenoid is vitamin A_1 (all-*trans*-retinol).

An important and quite widespread product of the catabolism of carotenoids is apocarotenal β-citraurin, (*R*)-3-hydroxy-8′-apo-β-carotene-8′-al with 30 carbon atoms in the molecule (**9-186**). Other important apocarotenals are bixin (*cis*-bixin) with 22 carbon atoms and crocetin with 20 carbon atoms.

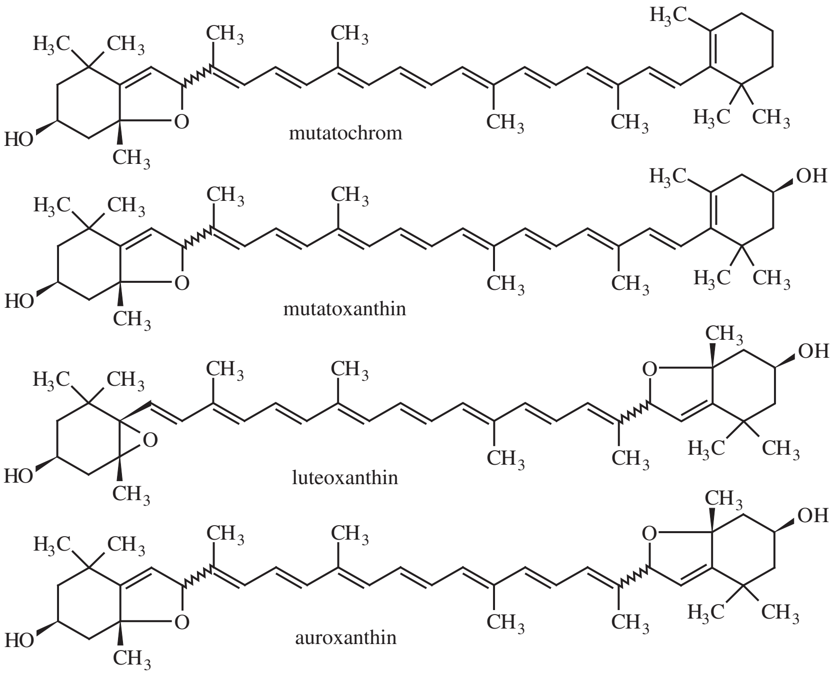
9.9.2 Occurrence

Carotenoids are important and are the most widespread lipophilic pigments of many fruits and vegetables. They occur in all photosynthetic plant tissues, where they are found as photochemically active components of plastids called chromoplasts. Carotenoids are often accompanied by other pigments, for example by anthocyanins in peaches and apricots. The presence of carotenoids in the green parts of plants is often masked by chlorophyll pigments.

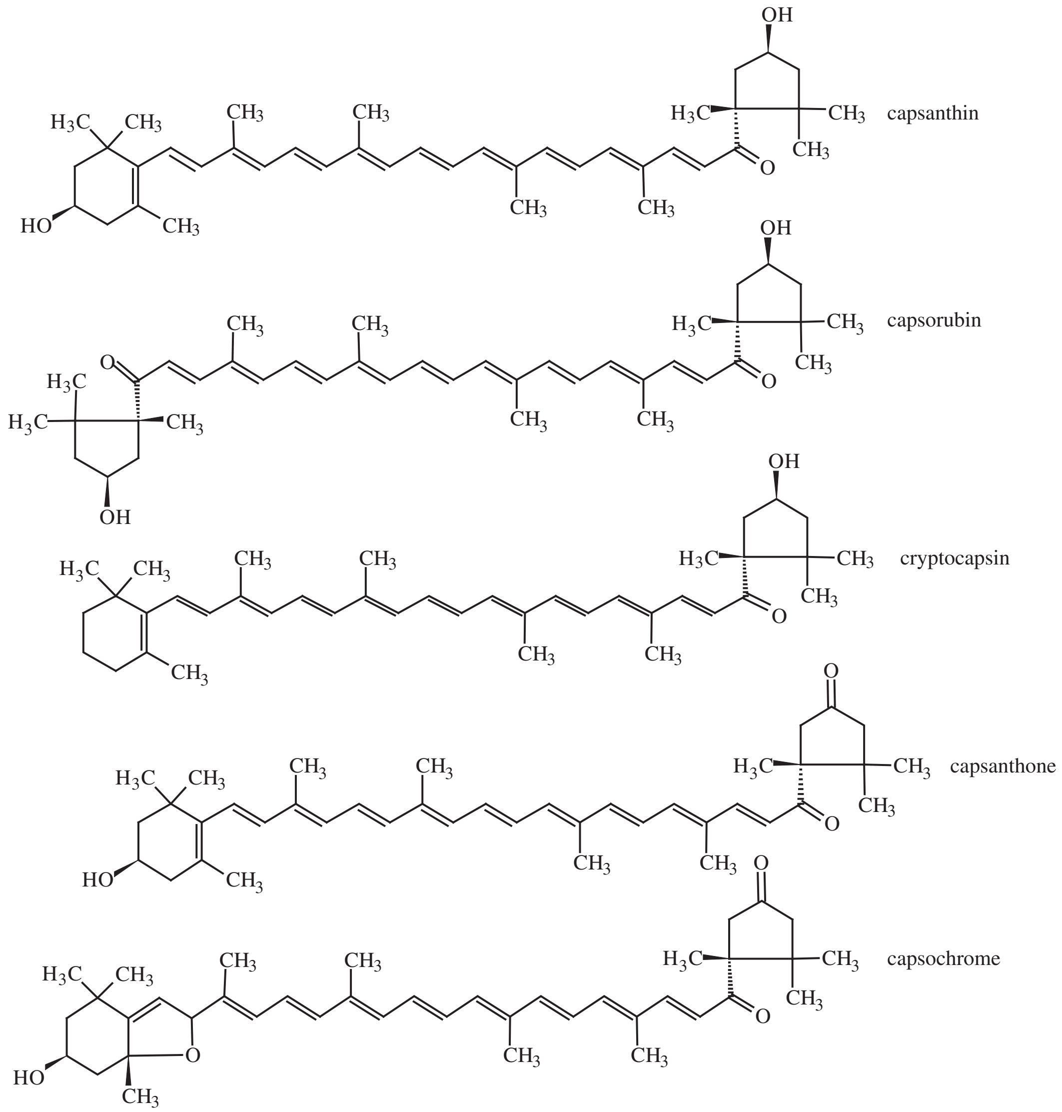
Qualitative and quantitative composition of carotenoids depends on many factors, such as the species and varieties of plants, season, maturity stage, method of processing and other factors. In some fruits and also in potatoes, carotenoids occur in units of mg/kg, but in most fruits and vegetables they are present in dozens of mg/kg, and in carrots, tomatoes and peppers there are hundreds of mg/kg of carotenoids.

A certain proportion of carotenoids in plants is associated with proteins, as is the case of chlorophyll pigments. These associates are generally known as **carotenoproteins**. Xanthophylls are also present as fatty acids esters or as glycosides. For example, the main yellow pigment of sunflower (*Helianthus annuus*, Asteraceae) flowering head is lutein dipalmitate.

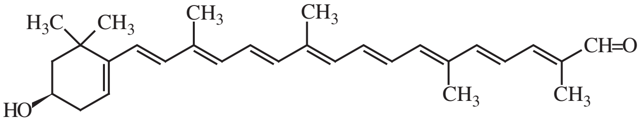
All-*trans*-isomers of carotenoids in fresh and thermally processed materials are accompanied by small amounts of *cis*-isomers, called **neocarotenoids**. β-Carotene is accompanied mainly by geometric isomers 9-*cis*, 13-*cis*- and 15,15′-*cis*-β-carotene. Lutein is accompanied mainly by 9-*cis*- and 9′-*cis*, 13-*cis*- and 13′-cis isomers; less common are 15-*cis* and 15′-*cis* isomers. Neoxanthin is accompanied by 9-*cis*-, 9′-*cis*-, 13-*cis*- and 13′-*cis* isomers. Thermal processing can induce carotenoid *trans* to *cis* isomerisation.



9-184, 5,8-epoxides



9-185, cyclopentanols and cyclopentanones



9-186, β-citraurin

9.9.2.1 Fruits

A particular type of fruit usually contains a number of carotenoids (see Table 9.14), of which some may be the dominating pigments. Rarely – for example in apricots and mango – the main pigment is β-carotene. Other pigments are various other carotenes and xanthophylls that are present in very small quantities. Peaches contain higher amounts of xanthophylls in comparison with apricots, and these partly occur in the form of fatty acid monoesters and diesters. The main fatty acids bound in xanthophylls are palmitic and myristic acids. Oranges contain relatively small amounts of carotenes, but highly variable amounts of cryptoxanthin, lutein, antheraxanthin and violaxanthin as the main pigments that are accompanied by other xanthophylls. β-Cryptoxanthin and zeaxanthin are the most abundant pigments of persimmon flesh (*Diopyros kaki*, Ebenaceae), and the total amount of these two components accounts for 38–85% of the total carotenoids.

9.9.2.2 Vegetables

9.9.2.2.1 Carrots

The predominant pigment of carrots is β-carotene (Table 9.14). Its content is generally 60 to 120 mg/kg, but the amount of β-carotene in some varieties may reach 300 mg/kg. These pigments are partly associated with proteins.

Table 9.14 Composition and content of main carotenoids of fruits and vegetables.

Carotenoids	Content (mg/kg fresh weight)							
	Apricot	Mango	Orange	Persimmon	Carrots	Spinach	Tomato	Bell pepper
Carotenes								
Lycopene	0.1	-	-	trace-1.1	-	-	16-750	-
Neurosporene	-	-	-	-	-	-	3.0	-
ζ-Carotene	0.4	trace-0.1	0.5	-	-	-	8.4	-
Phytofluene	0.3	-	1.3	-	-	-	5.1	-
Phytoene	0.6	-	0.4	-	-	-	6.0	-
β-Carotene	64	4.9-27	0.1-0.4	0.1-1.2	46-103	33-89	2.8-5.8	51-275
α-Carotene	-	-	0.1-0.2	trace-0.14	22-49	trace	-	-
γ-Carotene	0.2	-	-	-	6.3-27	-	0.4-1.6	-
Xanthophylls								
5,6-Epoxylycopene	-	-	-	-	-	-	5.3	-
β-Cryptoxanthin	-	-	0.1-7.1	0.6-9.4	-	-	-	36-79
α-Cryptoxanthin	-	0.3-1.1	-	-	-	-	-	-
Zeaxanthin	-	-	0.5	0.5-9.4	-	-	-	40-125
Lutein	-	-	0.3	Tr-0.6	1.1-5.6	42-81	0.4-1.3	
Antheraxanthin	-	-	0.6		-	-	-	33-44
Violaxanthin	-	0.7-3.0	0.7	0.1-0.9	-	74	-	53-98
Neoxanthin	-	-	-	0.1-0.7	-	24	-	174
Mutatochrom	-	trace-0.1	-	-	-	-	-	-
Mutatoxanthin	-	trace-2.0	0.6	-	-	5.0	-	164
Luteoxanthin	-	0.8-5.5	1.7	-	-	-	-	85
Auroxanthin	-	0.1-0.4	1.2	-	-	-	-	-
Capsanthin	-	-	-	-	-	-	-	523-1 207
5,6-Epoxycapsaxanthin	-	-	-	-	-	-	40-216	-
Capsorubin	-	-	-	-	-	-	53-179	-
Capsolutein	-	-	-	-	-	-	69-213	-
Cryptocapsin	-	-	-	-	-	-	814	-

9.9.2.2.2 Leafy vegetables

In leafy vegetables, β-carotene usually amounts to 10–20% of total carotenoid content. As in carrots, other carotenoid pigments are various xanthophylls. Lutein, violaxanthin and neoxanthin usually occur in large amounts, while cryptoxanthin, zeaxanthin (the main carotenoid of maize), antheraxanthin and other xanthophylls are found in smaller amounts (Table 9.14). The presence of carotenoids is masked by chlorophylls. Lettuce (*Lactuca sativa*, Asteraceae) is an example of a vegetable accumulating a higher amount of lactucaxanthin.

9.9.2.2.3 Tomatoes

The main pigment in tomato, lycopene, typically represents 85% of the tomato's total carotenoids. The content of β-carotene is relatively low, about 6 mg/kg, and about 1 mg/kg represents γ-carotene (see Table 9.14). Some orange hybrid tomatoes contain lower amounts of lycopene and higher amounts of β-carotene (up to about 80 mg/kg) and γ-carotene (about 7 mg/kg).

Lycopene content increases during tomato ripening and the final content is in the range of 30–200 mg/kg (about 95% represents the all-*trans* isomer). Tomato products are mostly prepared by

evaporation of tomato juice, therefore tomato products, such as concentrated juice (62 mg/kg), puree (133 mg/kg) or ketchup (102 mg/kg), contain higher concentrations of lycopene than the starting material (30 mg/kg), but also contain less stable *cis*-isomers, the content of which increases with temperature and time of heating. Up to 20% of the initially present *trans*-isomer may isomerise.

9.9.2.2.4 Bell peppers

Some carotenoids are found only in a limited number of plant species. An example is capsanthin and other cyclopentylketones in red bell peppers (*Capsicum* spp., Solanaceae) (Table 9.14).

The main pigments in green peppers, in addition to chlorophylls, are lutein (8–14 mg/kg), violaxanthin (8–10 mg/kg), neoxanthin (8–9 mg/kg) and β-carotene (6–8 mg/kg). During maturation, the minority yellow xanthophylls present in green fruits (β-cryptoxanthin and zeaxanthin) are enzymatically oxidised via 5,6-epoxides (e.g. via 5,6-epoxycryptoxanthin, antheraxanthin and violaxanthin) to several red κ-carotenes (Figure 9.21). The principal red pigment is always capsanthin, which constitutes 32–38% of carotenoid pigments of peppers. Present in amounts of 1–4% are capsanthin 5,6-epoxide, capsorubin (6–10%) and a small number of other substances, such as capsolutein, cryptocapsin and other pigments. The relative content of major carotenoids formed during ripening of pepper pods is given in Table 9.15.

Biosynthesis of κ-carotenes proceeds simultaneously with esterification of pigments by fatty acids. The majority of pigments of mature peppers (about 80%) are totally or partially esterified with fatty acids. The main fatty acids of yellow xanthophylls are linoleic, myristic and palmitic acids, in red xanthophylls they are mainly bound lauric, myristic and palmitic acids.

9.9.2.2.5 Rose hips

The total amount of carotenoids in rose hips from different species of the genus *Rosa* (Rosaceae) ranges from 101 to 834 mg/kg in flesh of fresh rose hips or from 42.3 to 1024 mg/kg (dry matter), of which 18.7–516 mg/kg represent carotenes, and 23.6–38.9 xanthophylls. The main pigment is lycopene (12.1–296 mg/kg), which is followed by other carotenes, (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene, also known as (9*Z*,9′*Z*)-ζ-carotene or prolycopene (1.2–21.3 mg/kg), γ-carotene (0–15.2 mg/kg) and ζ-carotene (0–14.6 mg/kg). The main xanthophyls are lutein and zeaxanthin (7.2–25.8 mg/kg), neochrome (6.6–9.8), neoxanthin (0–8.8 mg/kg) and violaxanthin (0–8.1 mg/kg).

Table 9.15 Changes of main carotenoids during pepper pods ripening (% of total carotenoids).

	Degree of ripeness					
Carotenoids	**Green**	**Light yellow**	**Yellow**	**Orange**	**Red**	**Dark red**
α-Carotene	0.3	0.7	0.4	0.5	0.2	0.2
β-Carotene	11.3	12.2	7.1	5.9	9.2	8.9
α-Cryptoxanthin	1.0	1.8	0.6	0.3	0.3	0.3
β-Cryptoxanthin	0.7	0.8	3.5	6.5	5.8	5.1
Zeaxanthin	6.7	6.8	10.5	19.5	16.9	15.3
Lutein	31.9	28.8	1.5	0.1	0.0	0.0
Antheraxanthin	2.3	0.3	1.4	3.6	1.4	1.6
Violaxanthin	4.8	1.4	6.3	4.2	1.3	2.0
Neoxanthin	3.7	5.5	4.0	0.0	0.0	0.0
Mutatoxanthins[a]	1.2	1.4	1.5	2.1	3.1	3.0
Luteoxanthins[a]	5.6	5.1	3.9	1.6	1.5	1.5
Auroxanthins[a]	0.1	0.8	0.2	0.0	0.0	0.0
Capsanthin	0.9	1.3	24.6	28.2	29.3	28.3
Capsorubin	0.0	0.0	2.8	2.7	2.0	2.6
Cryptocapsin	0.3	0.3	0.3	0.8	0.5	0.8
Capsanthon	0.0	0.0	0.1	0.4	0.4	0.4
Total content (mg/kg)	**115**	**168**	**448**	**1327**	**6107**	**9947**

[a]Mixture of two isomers.

9.9.2.3 Other plant materials

9.9.2.3.1 Cereals

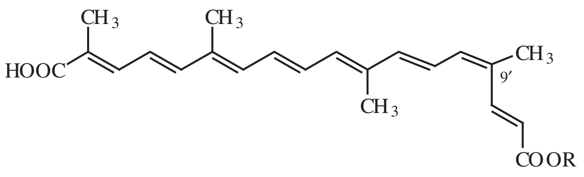
The common wheat varieties (*Triticum aestivum*, Poaceae) contain as the main carotenoid lutein (about 2 mg/kg), which is accompanied by small amounts of β-carotene (about 1% of total carotenoids) and traces of zeaxanthin. The total carotenoid content in *T. durum* is about three times higher than that in common wheat, with lutein (1.5 to 4 mg/kg) as the main component. Lutein is similarly the main carotenoid component in oats and barley.

Maize is exceptionally high in lutein at a concentration of about 20 mg/kg. In addition to the high content of lutein, maize also has high concentration of zeaxanthin (6–10 mg/kg), β-cryptoxanthin (2 mg/kg), β-carotene (1 mg/kg) and small concentrations of 15-*cis*-lutein, 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, 9′-*cis*-lutein and 9-*cis*-zeaxanthin.

The major brown rice carotenoids are β-carotene and lutein (both about 0.1 mg/kg), while zeaxanthin levels are lower (about 0.030 mg/kg).

9.9.2.3.2 Annatto

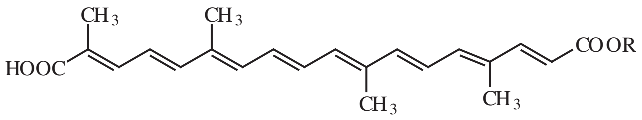
Annatto (also known as achiote) is the pigment from fruits (red seeds and pulp) of the achiote tree (*Bixa orellana*, Bixaceae), which is indigenous to Central and South America, where it is used as a dye, as medicine, and as an ingredient in many foods. The seeds can be ground into a powder, turned into a paste or infused into oil. The main natural component of annatto is apocarotenoid bixin, also known as (9′*Z*)-bixin (9′-*cis*-bixin, **9-187**) that arises as a product of lutein catabolism. The amount of bixin in seeds is about 2%. Bixin is orange, relatively unstable and slightly fat-soluble (it dissolves to give about 5% solutions).



9-187, (*Z*)-bixin, R = CH_3
(*Z*)-norbixin, R = H

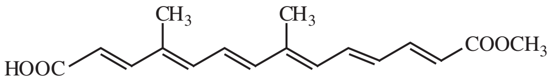
Commercially, the seeds and flesh are processed by extraction with vegetable oils at reduced pressure, and at temperatures lower than 130 °C. Under these conditions, 9′-*cis*-bixin undergoes partial isomerisation to the all-*trans* isomer called (*E*)-bixin (*trans*-bixin, **9-188**). Extracts contain from about 0.2 to 0.5% of a mixture of these two pigments in different proportions according to the conditions of extraction. The resulting *trans*-bixin is a red, relatively stable and fairly fat-soluble pigment.

The extraction of annatto seeds with aqueous alkaline solutions (at temperatures up to 70 °C) produces orange dicarboxylic acid called norbixin or 9′-*cis*-norbixin (**9-187**), which is soluble in a polar media. Isomerisation of norbixin yields red all-*trans*-norbixin (**9-188**), which is slightly soluble in fats.



9-188, (*E*)-bixin, R = CH_3
(*E*)-norbixin, R = H

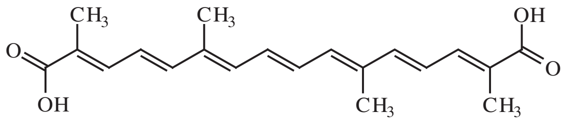
During extraction, 9′-*cis*-bixin is also partly decomposed to orange and pale yellow products. The main product is 14-methyl-hydrogen-4,8-dimethyltetradeca-2,4,6,8,10,12-hexaene-1,14-dioic acid (**9-189**). This compound exists in various stereoisomers such as the *all-trans* isomer, 15-*cis*, 13-*cis* and 9-*cis* isomer. Related compounds with 18 and 13 carbon atoms per molecule, *m*-xylene, toluene and other products also arise. Coloured degradation products of 9′-*cis*-bixin may constitute up to 40% of pigments in commercial products.



9-189, 14-methyl-hydrogen-4,8-dimethyltetradeca-2,4,6,8,10,12-hexaene-1, 14-dioic acid

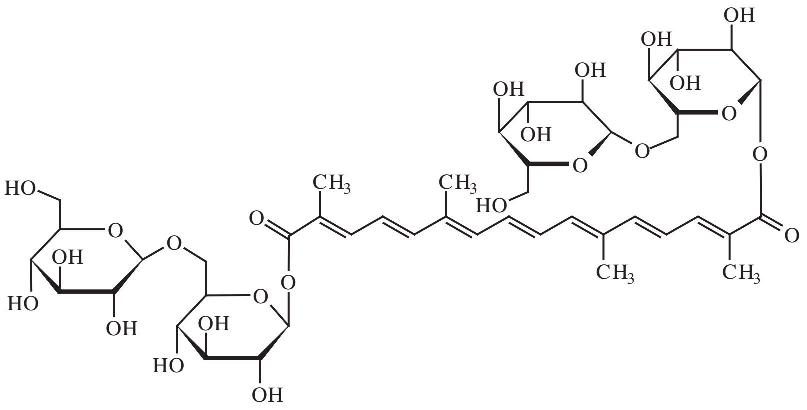
9.9.2.3.3 Saffron

Each flower of the saffron crocus (*Crocus sativus*, Iridaceae) has three filiform (20–30 mm long), purplish brown stigmas, which are dried and used as a culinary spice for its pungent, characteristic spice odour (the carrier is mainly aldehyde safranal, see Section 9.9.5.2.3), bitter taste (caused by picrocrocin, see Section 8.3.5.1.3) and colour. Saffron contains water-soluble yellow–orange apocarotenoid pigments, the chromophore of which is water-insoluble brick red aglycone crocetin, also known as α-crocetin (8,8′-diapocarotene-8,8′-dicarboxylic acid, **9-190**) formed by cleavage of zeaxanthin. Crocetin in saffron occurs as a water soluble yellow–orange ester of crocetin with disaccharide gentiobiose (di-β-gentiobiosylcrocetin), which is called α-crocin or just crocin (**9-191**). Crocin is accompanied by some minor pigments: di-β-gentiobiosyl-, β-gentiobiosyl-β-D-glucosyl-, di-β-D-glucosyl-, mono-β-gentiobiosyl- and mono-β-D-glucosylesters and also by esters derived from trisaccharide neapolitanose, such as β-gentiobiosyl-β-neapolitanosylcrocetin and di-β-neapolitanosylcrocetin.



9-190, crocetin

The disadvantage of saffron is its high price, so it is often falsified. Dry orange marigold petals (*Calendula officinalis*, Asteraceae) are mostly used for falsification, or alternatively the petals of safflower (*Carthamus tinctorius*, Asteraceae). The pigment of yellow marigold petals is 5,6-epoxylutein, also known as flavoxanthin, and the pigment of orange petals is lycopene. The pigment of safflower



9-191, crocin

petals is carthamin (see Section 9.4.2.5.1). Crocin is also found in fruits of the gardenia (*Gardenia augusta*, syn. *G. jasminoides*, Rubiaceae).

9.9.2.3.4 Vegetable oils

The concentration of carotenoids in crude vegetable oils is about 0.03–0.25%. Their content in refined oils is lower and depends on the conditions during refining. Pigments that are found in refined oils differ in their structure from natural pigments as they contain products of isomerisation and degradation of natural pigments.

A relatively high content of carotenoids (0.05–0.2%) is found in palm oil obtained from mesocarp of oil palm seeds (*Elaeis guineensis*, Arecaceae). It has a light orange colour as its main components are α- and β-carotene, occurring in a ratio of about 2 : 3.

9.9.2.4 Foods of animal origin

Carotenoids are similarly present in foods of animal origin. However, animals are unable to synthesise carotenoids *de novo*, and only convert plant pigments occurring in food into substances of different structure or store them as such.

9.9.2.4.1 Depot fats of mammals and birds

The main pigments of depot fats of birds (poultry) and mammals are xanthophylls lutein and zeaxanthin. Also present are small amounts of β-carotene and other pigments.

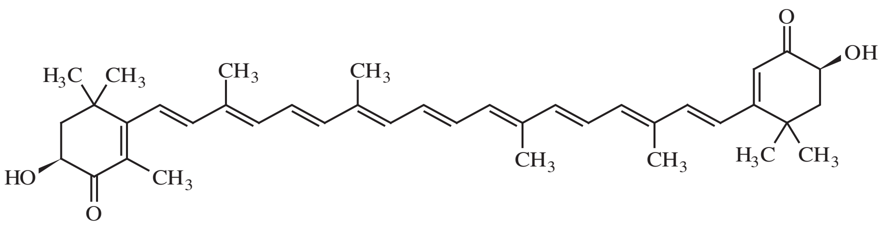
9.9.2.4.2 Eggs

Egg yolk and shell colours are important aspects of egg quality in many countries. Egg yolk pigmentation is a complex process influenced by many factors, such as nutrition, carotenoid source, health, egg handling and storage. Egg yolk contains as the main pigments the same xanthophylls and carotenes that occur in the depot fat of hens. The most abundant pigment is lutein/zeaxanthin, followed by β-cryptoxanthin and β-carotene and other carotenes occurring in the feed (such as canthaxanthin and β-apo-8′-carotenal). The amount of β-carotene is low (0.3–1% of total carotenoids), as this carotene is quickly metabolised.

Brown-shelled eggs presently dominate markets in the United Kingdom, France, Ireland and Portugal, whereas white eggs are preferred in Germany, Austria, Switzerland, Spain, the United States and Australia. The pigments of brown eggs are mixtures of protoporphyrin IX, biliverdin IX and its zinc chelate, with traces of other haemoglobin derived pigments in various proportions.

9.9.2.4.3 Fish and crustaceans

The red pigment of salmonid fish and many crustaceans (shrimps, crabs, crayfish and others) is xanthophyll astaxanthin, (3*S*,3*S*′)-astaxanthin, which is (3*S*,3*S*′)-β,β-carotene-3,3′-dihydroxy-4,4′-dione (**9-192**). Astaxanthin in crustaceans is bound to proteins in dark blue–red and green–red carotenoproteins. During cooking, proteins denature and the typical red colour of free astaxanthin becomes more vibrant. For example, the light blue crab *Homarus gammarus* (Nephropidae), known as European lobster



9-192, astaxanthin

or common lobster, contains the blue carotenoprotein known as α-crustacyanin. The protein (320 kDa) is composed of 16 apoprotein units, each of which contains one molecule of astaxanthin.

9.9.3 Use

Some carotenoid pigments are used as fresh or dried plant parts or extracts (so-called oleoresins) to impart colour to food (e.g. carrots, orange peels, tomatoes, saffron, annatto and paprika, which is made from dried and ground fruits of various *Capsicum* species and cultivars). Palm oil containing carotenoid pigments is also used for the same purpose.

Natural and synthetic carotenoids have found use as lipophilic and hydrophilic food dyes and also, relatively recently, as antioxidants. In amounts of 1–10 mg/kg, carotenoids are used to impart colour to many foods, such as margarines, cheeses, yoghurt, ice creams, fruit juices, dressings, flour, pasta products and cakes. The most common carotenoids are β-carotene (E160a), annato (E160b), paprika extract (E160c), lycopene (E160d), degradation product of β-carotene β-apo-8′-carotenal (E160), orange–red ethyl ester of the corresponding acid, which is ethyl β-apo-8′-carotenoate (Food Orange 7, E160f), lutein (E161b) and red canthaxanthin (E161g, **9-193**).

Canthaxanthin (β,β-carotene-3,3-dione) is also found in some beetle species and occurs as the major pigment in flamingo feathers, along with astaxanthin and other minor xanthophylls, such as phoenicoxanthin, (3-hydroxy-β,β-carotene-3,3-dione), phoenicopterone (β,ε-carotene-4-one) and echinenone.

The above listed carotenoids are also added to the feed of dairy cows and poultry to ensure desirable pigmentation of milk, dairy products, eggs and meat. All these carotenoids are added to feed for laying hens and broilers (to provide pigmentation of eggs and meat), with the exception of β-carotene, and also synthetic citranaxanthin (E161i, **9-194**) and carotenoids obtained from different plant materials. The reason why the feed for laying hens and broilers does not contain added β-carotene is its quick conversion into retinol, which has almost no effect on the yolk and meat colours. β-Carotene in beef cattle is stored in adipose tissue and also passes partly into milk.

9.9.4 Biochemistry, physiology and nutrition

Some carotenoids are precursors (provitamins) of vitamin A. Other carotenoids, such as cryptoxanthin, zeaxanthin and lutein, exhibit, by contrast, about half of the activity of provitamins A. Some carotenoids, such as lycopene, astaxanthin and canthaxanthin, are more effective in quenching singlet oxygen than β-carotene. Carotenoids also react with free radicals such as β-carotene. Because of their antioxidative properties, they are used in the prevention of degenerative processes and as anticancer agents.

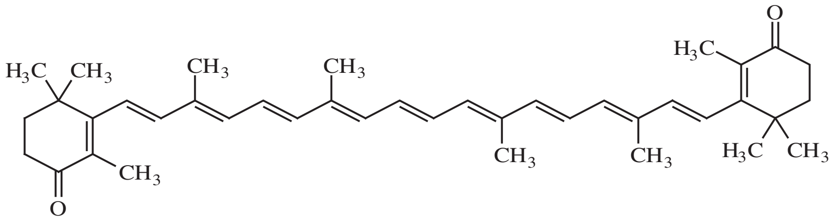
9.9.5 Reactions and changes

The combined effect of oxidoreductases, light, heat, oxygen, hydronium ions and other factors can lead to isomerisation, oxidation and degradation of carotenoids and xanthophylls (see Section 5.2.6.2). Carotenoids present in the form of carotenoproteins are more stable than free substances. Xanthophylls, especially epoxides of carotenoids, are more susceptible to changes under the conditions used during food processing. Apocarotenoids, which contain a carboxyl group, form soluble salts in alkaline media.

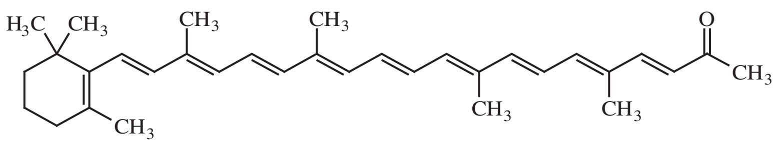
9.9.5.1 Carotenoids and colour

Food products, which undergo undesirable colour changes in the presence of enzymes and oxygen, are sterilised to achieve the inactivation of enzymes, and stored in an inert atmosphere or in the presence of antioxidants. In cases where it is impossible to prevent the degradation of carotenoids (e.g. during the storage of flour or the manufacture of pasta, losses of carotenoids can reach 30–60%), the material can be coloured using synthetic carotenoids.

In acidic citrus juices, spontaneous conversion of 5,6- and 5′,6′-epoxides to 5,8- and 5′,8′-epoxides occurs. Relatively rapid



9-193, canthaxanthin



9-194, citranaxanthin

enzymatic degradation of carotenoids (about 50% of pigments are degraded within 20 min) occurs in stored raw spinach puree. Lipoxygenases are indirectly involved in the degradation of carotenoids, as a result of their reaction with free hydroperoxide radicals. Therefore, it is necessary to inhibit oxidoreductases of spinach leaves by blanching them in hot water before freezing. During storage of frozen blanched spinach puree, changes in the content of carotenes and xanthophylls are minimal.

Thermal processing of vegetables using mild processes, such as steaming, results in relatively low losses in the content of carotenes (up to 10%) and in somewhat higher losses in the content of xanthophylls. Epoxy xanthophylls, such as violaxanthin and taraxanthin, however, undergo significant losses. When cooking spinach, broccoli or green beans by steaming or boiling in water for 3–10 min, the loss of taraxanthin can reach 35–100%, and the loss of violaxanthin may be 35–65% depending on the type and method of processing. Cooking beans in water for 10 min results in a loss of about 60% and cooking for 1 hour leads to complete degradation of both epoxy carotenoids.

The pigment content during drying and grinding of pepper pods is reduced to about half. Most losses are caused by dilution of the product by non-coloured parts of fruits, such as seeds, but some degradation of carotenoids also occurs. The least stable are the yellow pigments, especially β-carotene, while the red pigments (capsanthin and its derivatives) only decompose to a small extent. In paprika stored in the presence of oxygen, the hydroxyl groups of cyclopentane ring of capsanthin are readily oxidised with the formation of the corresponding diketone capsanthone, also known as capsanthinone, which is (3*R*,5′*R*)-3-hydroxy-β,κ-carotene-3′,6′-dione. Capsanthone decomposes further to β-citraurin, β-citraurin yields 3-oxo-β-apo-8′-carotene, which is degraded to low molecular weight products. Products containing a carbonyl group in the molecule enter the non-enzymatic browning reactions, which results in a change of the red colour of paprika to brown.

Similar reactions also occur in dried and concentrated tomato products (such as dried soups, juice concentrates, pickles and ketchup). From 5,6-epoxylycopene (5,6-epoxy-5,6-dihydrolycopene) present in fresh fruits arises, through epoxide ring opening under acidic conditions, 5,6-dihydroxy-5,6-dihydrolycopen.

In stored foods containing natural bixin (9′-*cis* isomer), in the presence of light, all-*trans*-bixin may result and, by its hydrolysis, all-*trans*-norbixin. In cheeses containing bixin, these substances bind to phosphoproteins (e.g. to some caseins), causing pink discoloration of products. In addition to *trans*-isomers, some degradation products of bixin may also arise, such as methyl ester of all-*trans*-4,8-dimethyltetradeca-2,4,6,8,10,12-hexaenoic acid and aromatic hydrocarbons (e.g. *m*-xylene).

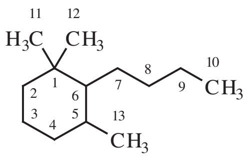
9.9.5.2 Carotenoids and aroma

Carotenoids are precursors of many important compounds, which are formed as products of their catabolism or oxidation, for example, during fruit ripening or processing. Oxidation of carotenoids into fragments called **apocarotenoids** or **diapocarotenoids** is provided by a group of enzymes known as dioxygenases, which catalyse the transfer of molecular oxygen to the substrate. Cleavage of carotenoids in the central double bond (so called symmetric fission) results in the formation of two C_{20} molecules of apocarotenoids. In this way, for example, the visual signalling molecules of retinal and retinoic acid is produced (see Section 5.2.2). Asymmetric (eccentric) cleavage of carotenoids yields apocarotenoid molecules with different carbon chain lengths. The most important degradation products are C_{15}, C_{13}, C_{11}, C_{10} and C_9 compounds. The biologically important C_{15} compound is phytohormone abscisic acid, which is regarded as the major player in mediating the adaptation of plants to stress and preparing plants for a period of dormancy (loss of leaves and other phenomena). Degradation products of carotenoids responsible for the smell and taste of food (mainly fruits and vegetables) and the smell of many flowers are mainly C_{13}, C_{11}, C_{10} and C_9 compounds, but C_{14}, C_8 and other apocarotenoids may also arise. The most important apocarotenoids are C_{13} compounds. Degraded carotenoids with more original carbons (C_{39}, C_{38} or C_{37}), called **norcarotenoids**, are not precursors of aromatic compounds. One to three carbon atoms are formally removed by other reactions (such as oxidation and decarboxylation) than is the case of apocarotenoids.

9.9.5.2.1 Apokarotenoids C_{13}

Apokarotenoids C_{13} result from the cleavage of C-9/C-10 and C-9′/C-10′ bonds. In the latter case, another fission product is a C_{14} diapocarotenoid. The main pigment of red varieties of tomatoes is lycopene (ψ,ψ-carotene), which is accompanied by other acyclic carotenes. Fission of the C-9/C-10 (C-9′/C-10′) bond in lycopene yields the odorous compound of tomatoes (3*E*,5*E*)-6,10-dimethylundeca-3,5,9-trien-2-one (pseudoionone) and, obviously, C_{14} diapocarotene 4,9-dimethyldodeca-2,4,6,8,10-pentaenedial. Acyclic carotene phytoene gives rise to C_{13} methylketone (*E*)-6,10-dimethylundeca-5,9-dien-2-one (known as geranylacetone or dihydropseudoionone) and diapocarotenoid 4,9-dimethyldodeca-4,6,8-trienedial (Figure 9.22). One molecule of geranylacetone may arise from ζ-carotene.

The C-9/C-10 (or C-9′/C-10′) double bond cleavage in alicyclic carotenes and xanthophylls yields products with a structure derived from the hydrocarbon megastigmane (**9-195**). Common carotenes may produce eight C_{13} methylketones (**9-196**). For example, the cleavage of β,β-carotene (β-carotene), β,ε-carotene (α-carotene) and (*R*)-β,β-carotene-3-ol (β-cryptoxanthin) produces β-ionone, which is a fragrant component of raspberries, blueberries, passion fruit, apricot, carambola, cherry, mango, plums, black tea, tomatoes, carrots, bell peppers and tobacco. α-Carotene is a precursor of (*R*)-ionone, also known as (+)-α-ionone. This compound, with a fruity and floral aroma reminiscent of violets and raspberries, is a fragrant component of blackcurrants, blueberries, raspberries,



9-195, megastigmane

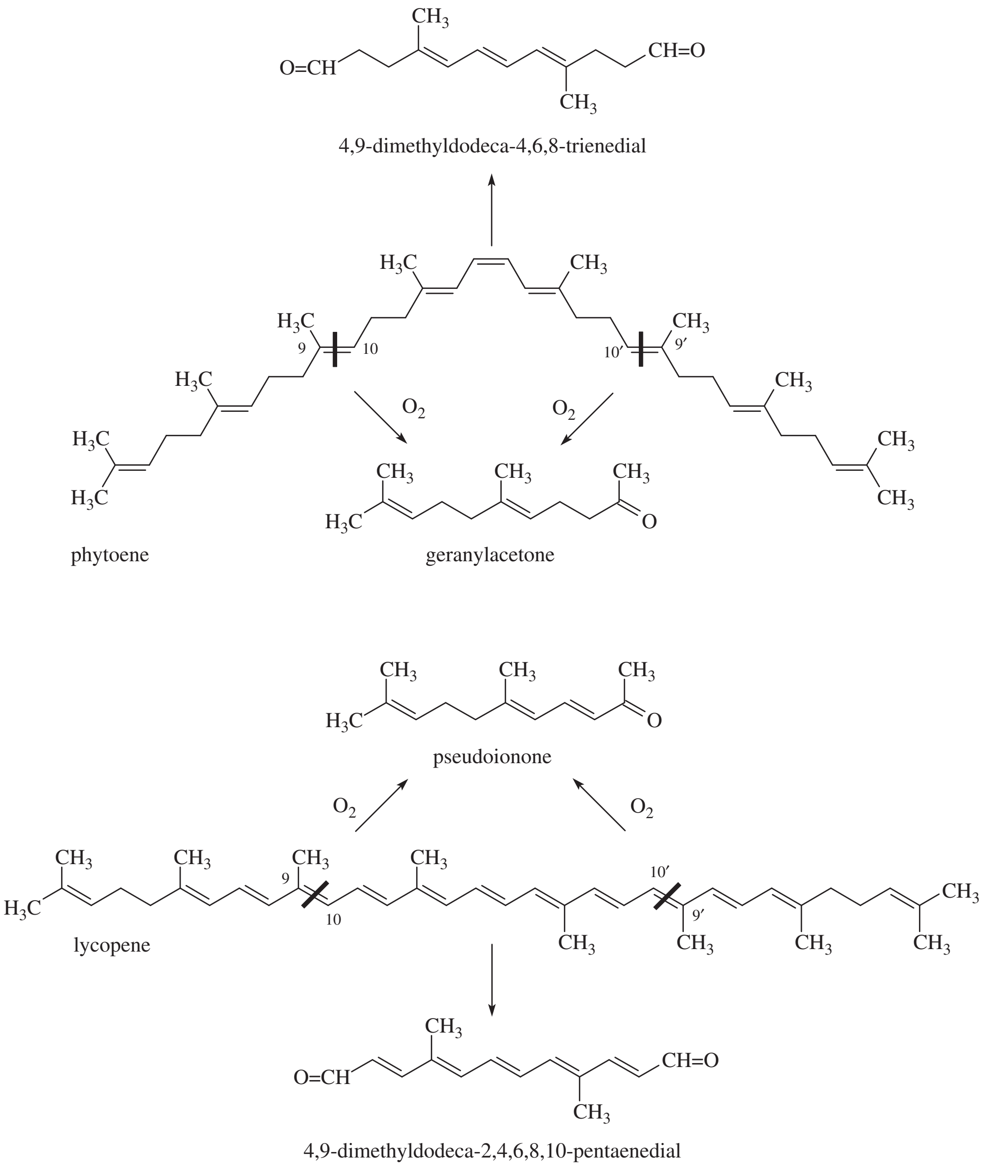


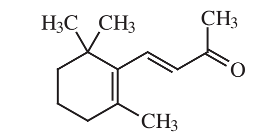
Figure 9.22 Fission of acyclic carotenes.

bananas, cherries, plums, peaches, vanilla, tomatoes, carrots, celery, black tea, tobacco and other products.

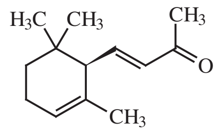
Hydroxy ionones and epoxy ionones arise by fission of various xanthophylls. For example, (*S*)-3-hydroxy-β-ionone arises from β-cryptoxanthin, (3*R*,3′*R*)-β,β-carotene-3,3′diol (zeaxanthin) and (3*R*, 3*R*′,6′*R*)-β,ε-carotene-3,3′diol (lutein). 5,6-Epoxy-β-ionone arises, for example, from (3*R*,5*S*,6*S*,3′*R*)-5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol (antheraxanthin) and (3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*S*)-5,6,5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-caroten-3,3′-diol (violaxanthin). (3*R*,6*R*)-3-Hydroxy-α-ionone may arise from lutein, (3*S*,6*S*)-3-hydroxy-α-ionone from (3*S*,3′*S*,6*S*,6′*S*)-ε,ε-carotene-3,3′-diol (lactucaxanthin) and (3*S*,6*R*)-3-hydroxy-α-ionone from (3*R*,3*S*′,6′*R*)-β,ε-carotene-3,3′-diol (3′-epilutein).

The special ketone known as grasshopper ketone arises from (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*R*)-5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol (neoxanthin). While maintaining the stereochemistry at carbon C-3, hydroxy ionones and grasshopper ketone can be glycosylated and become precursors of many other compounds. About 50 products formed by oxidation, reduction, dehydration and cyclisation have been identified; some of them being formed by more complex mechanisms. Some of these reactions are shown in Figure 9.23, which illustrates the conversion of (*S*)-3-hydroxy-β-ionone into 1,2-dihydro-1,1,6-trimethylnaphthalene. In small amounts, this compound is a fragrant component of peaches, strawberries, tomatoes and wine. It has a low threshold (20 μg/l in wine) and in older vintages of Riesling wines may be responsible for the kerosene-like smell, while in passion fruit juices it causes an off-flavour described as aroma flattening during pasteurisation.

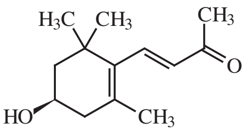
Figure 9.24 illustrates the mechanism of transformation of (3*S*,6*R*)-3-hydroxy-α-ionone into several very important



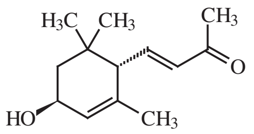
β-ionone



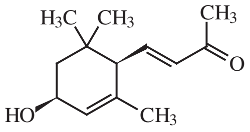
(6*R*)-α-ionone



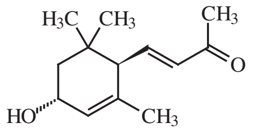
(3*S*)-3-hydroxy-β-ionone



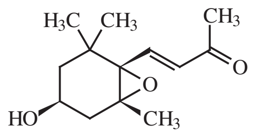
(3*S*,6*S*)-3-hydroxy-β-ionone



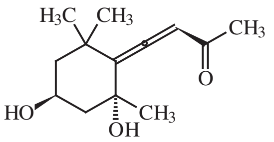
(3*S*,6*R*)-3-hydroxy-β-ionone



(3*R*,6*R*)-3-hydroxy-β-ionone



(3*S*,5*R*,6*S*)-5,6-epoxy-3-hydroxy-β-ionone



(3*S*,5*R*,6*R*)-grasshopper ketone

9-196, fission products of alicyclic carotenes and xanthophylls

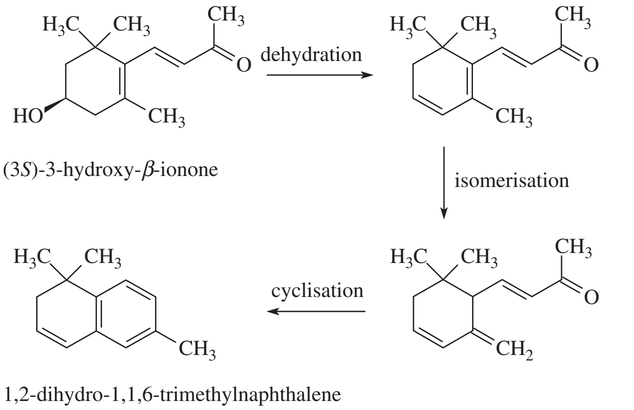
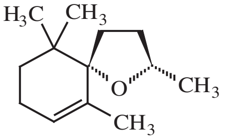


Figure 9.23 Formation of 1,2-dihydro-1,1,6-trimethylnaphthalene from (*S*)-3-hydroxy-β-ionone.

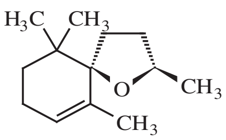
odour-active compounds, 3-oxo-α-ionone and 3,4-dihydro-3-oxoedulanes (components of wine aroma), a decalin derivative (a component of passion fruit aroma) and megastigmatrienones considered essential fragrances of tobacco.

Several important C_{13} compounds are derived from epoxy apocarotenoids. These epoxides yield, by epoxy group protonation and C-4 deprotonation, C-6 alcohols (epoxy apocarotenoids with hydroxyl group in the side chain give rise to diols), which may be glycosylated and, for example, hydrolysed during fruit ripening to the corresponding alcohols. An example is oxidation of 5,6-epoxy-3-hydroxy-β-ionone to dehydrovomifoliol, which is reduced to blumenol A, from which arises blumenol B and subsequently the important fragrance compound theaspirone. Theaspirone occurs in passion fruit, black tea and tobacco (Figure 9.25).

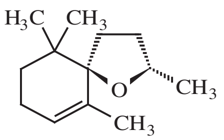
Other spiroethers (8,9-dehydrotheaspirones) may arise analogously from 7,8-dehydrovomifoliol via 7,8-dihydrovomifoliol by reduction of two double bonds. 7,8-Dihydrovomifoliol is found in fruits and vegetables in the form of glycosides. The (*S*)-7,8-dehydrovomifoliol has a fruity and flowery odour, but the (*R*)-isomer has an odour resembling wood. Dehydrovomifoliol and blumenol A may also be formed by singlet oxygen oxidation of 3-oxo-β-ionone. Structurally similar theaspiranes are important odorous components of many fruits (strawberries, blackberries, grapes, passion fruit, guava and others) and black tea. (2*S*,5*S*)-Isomers (**9-197**) and (2*R*,5*R*)-isomers (**9-197**) have a slight smell resembling camphor, the (2*S*,5*R*)-isomer has the smell of camphor and naphthalene, but the (2*R*,5*S*)-isomer has a very attractive fruity odour reminiscent of blackcurrants. Thanks to the enantioselectivity of biochemical mechanisms, some chiral compounds



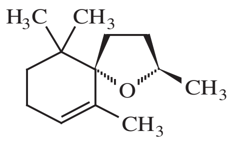
(2*S*,5*S*)-theaspirane



(2*R*,5*R*)-theaspirane



(2*S*,5*R*)-theaspirane



(2*R*,5*S*)-theaspirane

9-197, theaspiranes

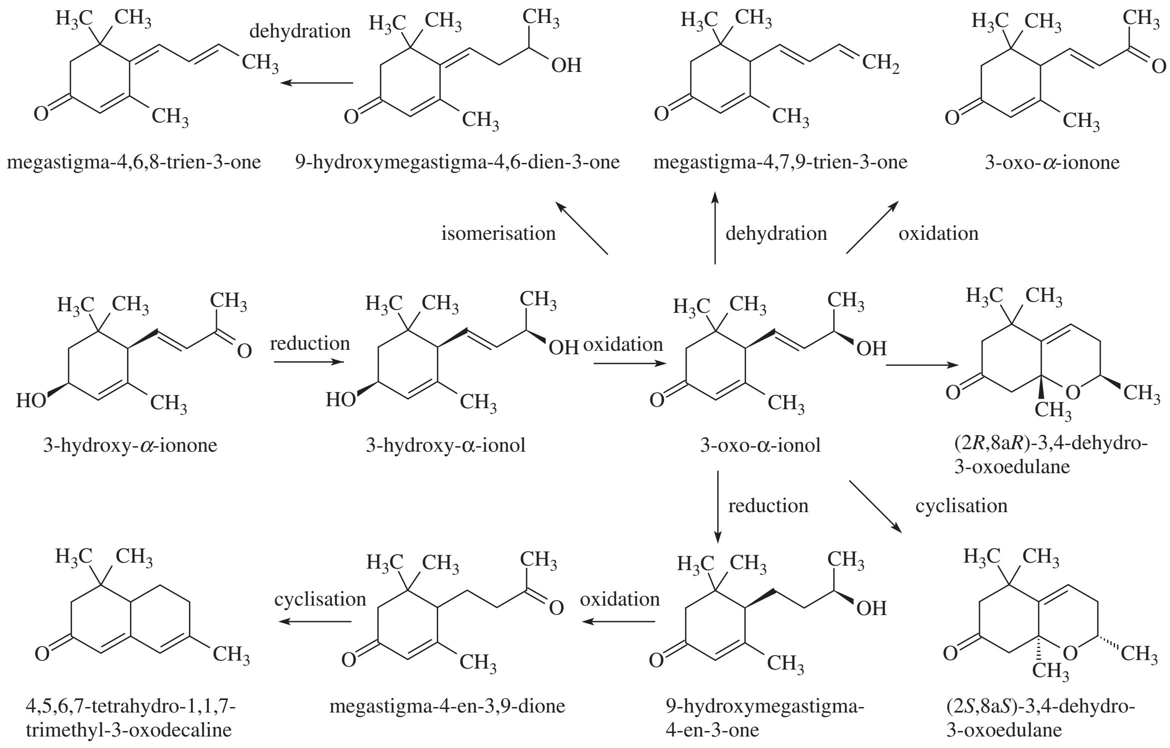


Figure 9.24 Formation of odorous compounds from (3*S*,6*R*)-3-hydroxy-α-ionone.

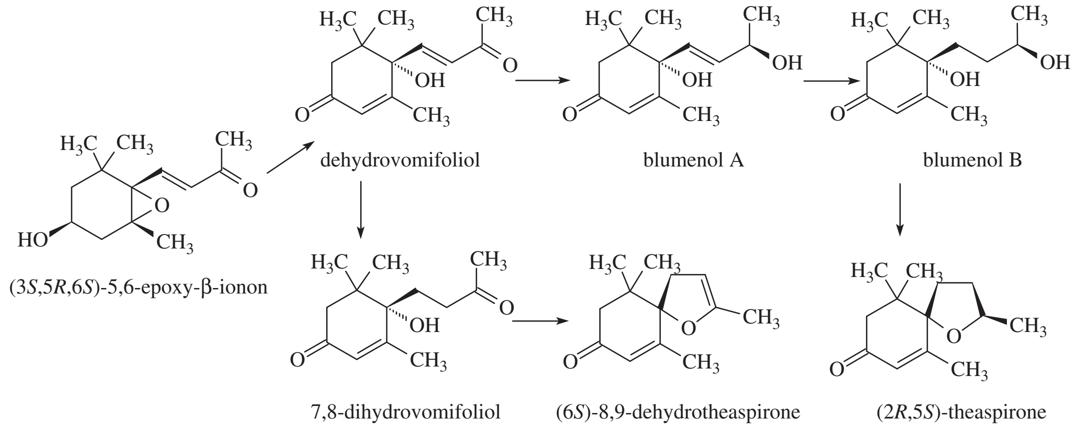
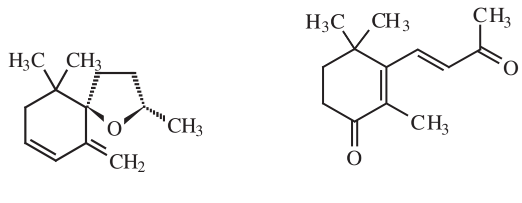


Figure 9.25 Formation of odorous compounds from epoxycarotenoids.

predominate in certain plant materials. For example, in green tea there is about 85% of (2*S*)-theaspiranes and about 15% of (2*R*)-theaspiranes. Related vitispirans (**9-198**) occurring in black tea and tobacco are important components of the aroma of grapes and wines. 4-Oxo-β-ionone (**9-199**) is formed by photooxidation of α-ionone.

The C_{13} damascones oxidised on carbon C-7 are very important fragrant compounds. β-Damascol, as a racemate, is an odorous component of papaya, black tea, rum and tobacco. It arises by oxidation of β-ionol with singlet oxygen (Figure 9.26) via β-damascone, which is reduced to β-damascol. α-Ionol may analogously be produced by oxidation of α-damascone. Its aroma resembles roses



9-198, (2*S*,5*R*)-vitispirane **9-199**, 4-oxo-β-ionone

and fruits. The precursor of related fragrant substance (*E*)-β-damascenone is grasshopper ketone formed by the cleavage of neoxanthin. Grasshopper ketone (Figure 9.27) is found in many fruits and vegetables (apricots, star fruit, grapes, kiwi, mango,

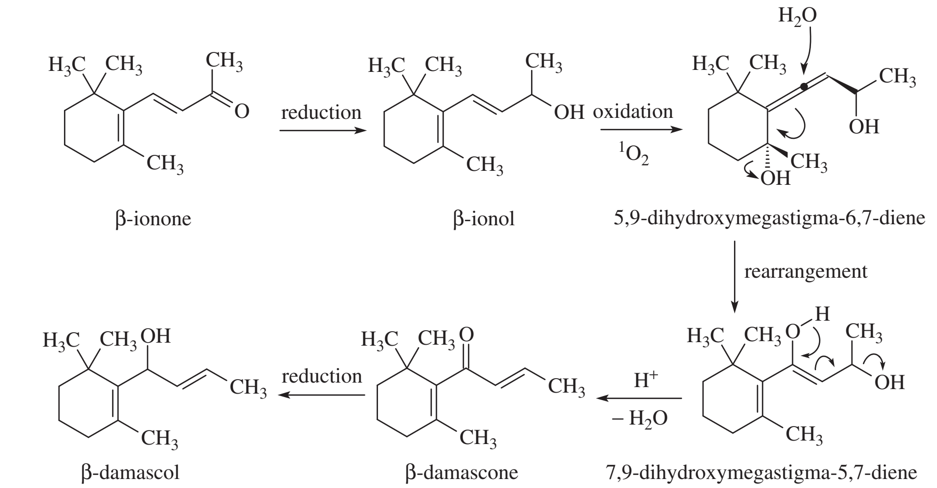


Figure 9.26 Formation of β-damascone and β-damascol from β-ionone.

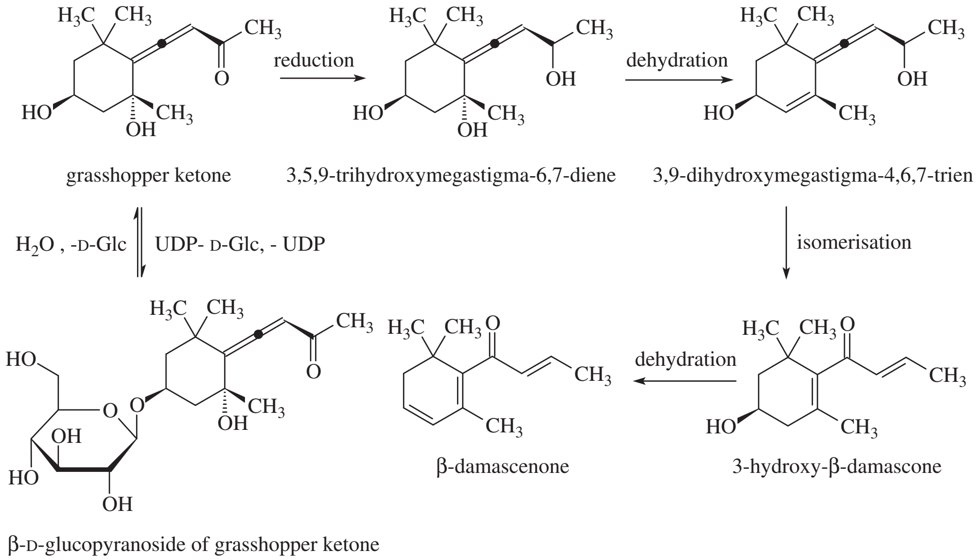


Figure 9.27 Formation of β-damascenone from grasshopper ketone.

apples, raspberries and tomatoes) and other foodstuffs (coffee, black tea, honey, beer, wine, rum and brandy). Hydroxylated damascones (such as grasshopper ketone and 3-hydroxy-β-damascenone) and other C_{13} alcohols are commonly found in plants as glycosides, the aglycones of which are released by enzymatic or acid hydrolysis. For example, glycosides present in grapes are hydrolysed during must production and wine fermentation.

9.9.5.2.2 Apocarotenoids C_{11}

An important compound of this group of apocarotenoids is the C_{11} apocarotenoid dihydroactindiolide presenting a cooling effect in the oral cavity. Dihydroactindiolide typically occurs as a constituent of tomato, black tea and tobacco odour. It is also a component of cassia oil, which is the oil of Chinese cinnamon called cassia (*Cinnamomum aromaticum*, syn. *C. cassia*, *Lauraceae*), and the wax obtained from sperm whale (see Section 3.4.1.3.1). Dihydroactindiolide is formed by oxidation of β-carotene, β-ionone and β-ionol with singlet oxygen (Figure 9.28).

9.9.5.2.3 Apokarotenoids C_{10}

Cleavage of carotenoids in the C-7/C-8 (C-7′/C-8′) double bond gives products, which are formally monoterpenoids. Cleavage of β-carotene yields 2,6,6-trimethylcyclohex-1-ene-1-carbaldehyde, which is known as β-cyclocitral (**9-200**). Citrocitral has been found in apricots, sugar melons *(Cucumis melo)*, watermelon (*Citrullus lanatus*, Cucurbitacaeae), tomatoes, bell peppers, peas, broccoli, black tea, rum and other materials. The best known aromatic substance of this group of apocarotenoids is safranal (4,5-dehydro-β-cyclocitral, **9-201**) with the smell and taste of saffron (see Section 9.9.2.3.2). Safranal arises by hydrolysis of a bitter tasting glycoside

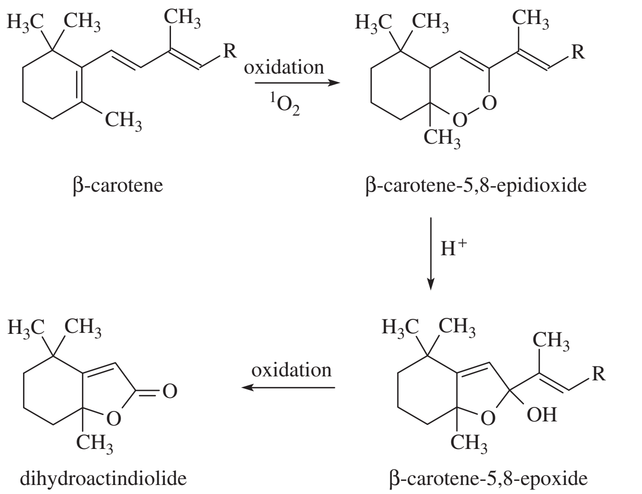
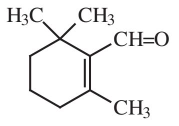
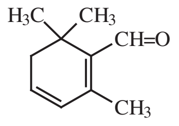


Figure 9.28 Formation of dihydroactindiolide from β-carotene.

picrocrocin and by dehydration of aglycone (*R*)-4-hydroxy-β-cyclocitral, respectively. Safranal is also a minor component of grapefruit juice, black tea and bell pepper aroma.



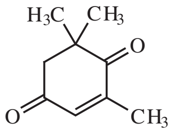
9-200, β-cyclocitral



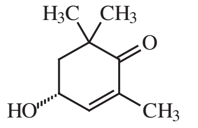
9-201, safranal

9.9.5.2.4 Apokarotenoids C_9 and C_8

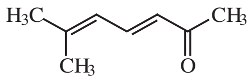
The main formation pathway of C_9 apocarotenoids with cyclohexanone and cyclohexenone structures is conversion of hydroperoxides derived from β-damascol (Figure 9.29). Hydroperoxides generated by autoxidation of carotenoids are further oxidised, reduced and hydrated to form a variety of different structures. The most important compound of this apocarotenoid group is probably 2,6,6-trimethylcyclohex-2-en-1,4-dione (4-oxoisophorone, **9-202**), whose scent recalls dry straw. The starting compound is apparently (*R*)-3-hydroxy-β-damascol that is oxidised to (*R*)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-one (**9-203**), oxidation of which yields 4-oxoisophorone. It occurs in black tea, saffron and tobacco. Acyclic carotene dehydrolycopene gives rise to methylketone (3*E*,5*E*)-6-methylhepta-3,5-dien-2-one (**9-204**), which is an aroma constituent of tomatoes.



9-202, 2,6,6-trimethylcyclohex-2-en-1,4-dione

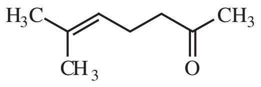


9-203, (*R*)-4-hydroxy-2,6,6-trimethylcyclohex-2-en-1-one



9-204, (3*E*,5*E*)-6-methylhepta-3,5-dien-2-one

An example of C_8 apocarotenoids occurring, for example, in tomatoes, is methylketone 6-methylhept-5-en-2-one (**9-205**), which is produced by C-5/C-6 (C-5′/C-6′) double bond cleavage of acyclic carotene lycopene. It may also arise from phytoene.



9-205, 6-methylhept-5-en-2-one

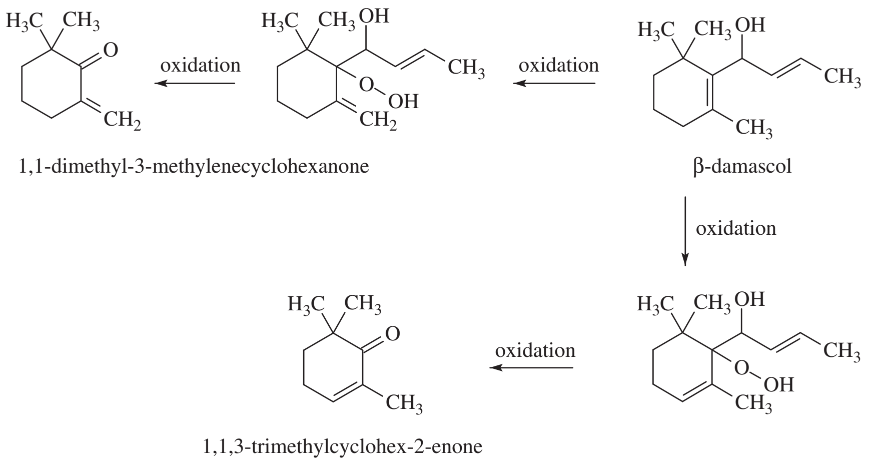
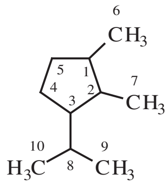


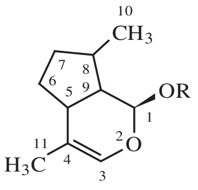
Figure 9.29 Formation of cyclohexanones and cyclohexenones.

9.10 Iridoids

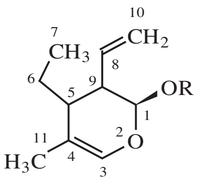
Iridoid monoterpenes called **iridoids** are a group derived from the skeleton of the hydrocarbon iridane (**9-206**). Their usual structure is represented by the formula **9-207.** The opening of a cyclopentane ring and introduction of various functional groups (mainly by oxidation) yields **secoiridoids** (**9-208**), which include many bitter substances of plants.



9-206, iridane



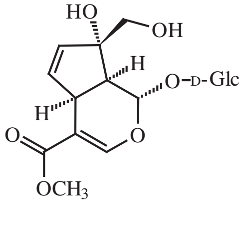
9-207, iridoids (basic structure)



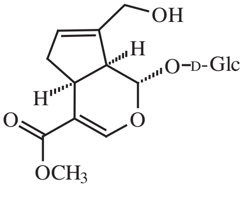
9-208, secoiridoids (basic structure)

Pigments derived from yellow to red fruits of common gardenia, also known as cape jasmine (*Gardenia augusta*, syn. *G. jasminoides*, Rubiaceae), an evergreen shrub growing in India, China and Japan, are iridoid pigments, important for their use as dyes in the textile industry and also for imparting colour to foods. Fruits located in pods contain three main groups of yellow and orange pigments: iridoid pigments, apocarotenoids (crocin and crocetin such as saffron) and flavonoids. Interest in the gardenia pigment was initiated in Japan through the pursuit of substitution of annatto and saffron pigments with cheaper pigments in cases where the specific flavour of the original materials was not required.

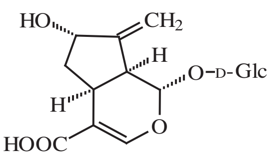
The main iridoid pigments of gardenia are the glucosides gardenoside (**9-209**), geniposide (**9-210**) and related compounds gardoside (**9-211**) and shanzhiside (**9-212**). They are accompanied by a number of related minor pigments (**9-213**). Their biosynthesis is based on geranyl diphosphate, and proceeds via (1*R*,2*S*,5*R*,8*S*)-iridodial (8-epiiridodial) and other intermediates.



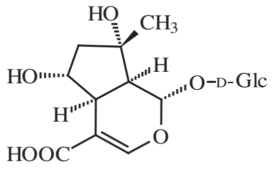
9-209, gardenoside



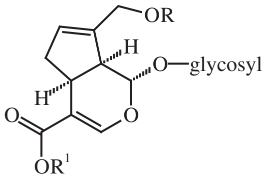
9-210, geniposide



9-211, gardoside



9-212, shanzhiside



9-213, minor gardenia iridoid pigments
geniposidic acid, $R = R^1 = H$, glycosyl = β-D-glucosyl
acetylgeniposide, $R = COCH_3$, $R^1 = H$, glycosyl = β-D-glucosyl
genipin gentiobioside, $R = H$, $R^1 = CH_3$, glycosyl = β-gentiobiosyl

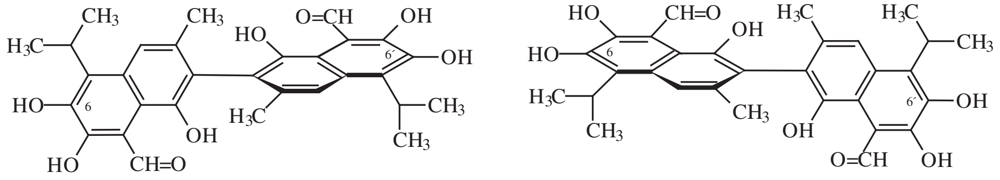
Preparation of pigments is based on their extraction with water, enzymatic hydrolysis of glycosides and reaction of aglycones with amines. By adjusting the process conditions (temperature, pH, oxygen concentration, type of reacting amino compounds and presence of metal ions) a number of pigments can be obtained whose colour is yellow-to-green, red, purple or blue, which is a combination of colours of the pigments produced. Gardenia pigments can be used to impart colour to ice creams, confectionery, pasta products and other foods.

9.11 Other terpenoid pigments

The toxic yellow pigment gossypol, 2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methyl)naphthalene, resembles phenolic pigments, but its biosynthetic precursor is a sesquiterpene hydrocarbon (+)-δ-cadinene, with the chemical name (1*S*,8a*R*)-1-isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene (**8-8**). In a sequence of oxidation reactions, δ-cadinene is transformed into the gossypol intermediate 4-formyl-1-isopropyl-2,3,5,7-tetrahydroxynaphthalene, which is known as hemigossypol. Further one-electron oxidation of hemigossypol gives rise to a C-5 radical, the resonance form (C-6) of which dimerises to gossypol.

Gossypol occurs in buds and seeds of upland (Mexican) cotton (*Gossypium hirsutum*, Malvaceae) and in other *Gossypium* species (such as *G. herbaceum*). Cotton seeds usually contain 0.6–1.5% of gossypol localised in special pigment cells. Gossypol occurs in two enantiomers, as (+)-gossypol and (−)-gossypol (**9-214**), which are in the ratio of 3 : 2. Currently used cultivated cotton varieties lack these pigment cells and have a low content of gossypol. In some varieties of cotton, 6-methoxygossypol and 6,6′-dimethoxygossypol (**9-214**) are found as minor products. During cottonseed oil extraction, gossypol passes from the seeds to the oil and is present in flour, used in some countries for human nutrition, and in meal, used as feed for poultry and livestock.

Gossypol is a very reactive compound (Figure 9.30) forming salts with metal cations, which involves hydroxyl groups at C-1 and



(+)-gossypol (–)-gossypol

9-214, gossypol

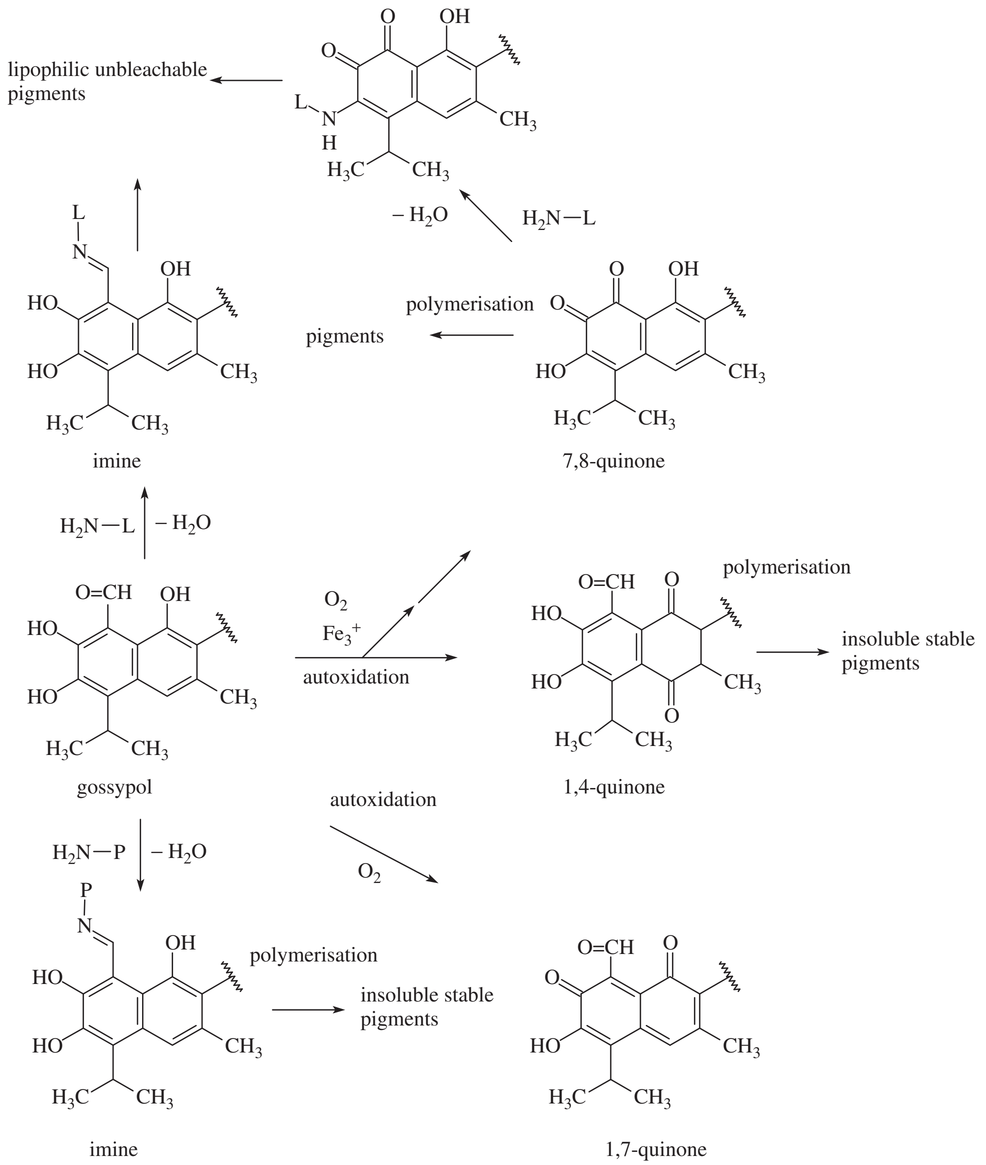


Figure 9.30 Reactions of gossypol (H_2N–P = protein with bound lysine, H_2N–L = phospholipid with bound ethanolamine).

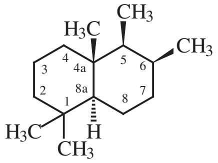
C-1′. By oxidation with oxygen or with ferric ions, the corresponding *o*- and *p*-naphthoquinones arise, which mutually polymerise and react with proteins and phospholipids with the formation of dark pigments. In scavenging free radicals, gossypol shows greater activity than both methylated derivatives.

The reaction of gossypol with proteins has been used to detoxify cottonseed oil. Gossypol in milled seeds reacts with seed globulins to form hydrophilic and hydrophobic pigments. The detoxifying effect is increased by seed steaming with the addition of ferrous sulfate, which catalyses decarbonylation of gossypol to

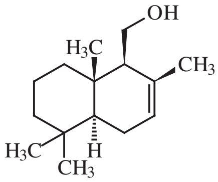
apogossypol, which is not stable and is less toxic than gossypol. Thus detoxified material contains 0.6–0.9% bound gossypol and 0.01–0.02% free gossypol. Small quantities of gossypol in oil react (either directly or after oxidation to quinone) with amino groups of phospholipids to form imines, which polymerise to fat-soluble macromolecular dark pigments, which cannot be effectively removed during subsequent refining.

Only (−)-gossypol is toxic, and only for omnivores. Chronic intoxication caused by low, long-term income is reflected in reduced appetite, liver damage and reduced blood clotting, and a reversible sterility in males at a dose of about 10 mg per day. In addition to these effects, gossypol has some activity as an anti-malarial drug and is studied for its anticarcinogenic properties.

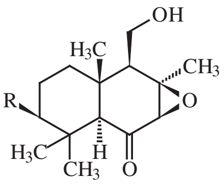
The colours of some higher fungi of the genus *Lactarius*, collectively known as milk caps, and of the closely related genus *Russula* (Russulaceae), are based on sesquiterpenoid pigments. The colour of the injured flesh as well as of the latex of several mushroom species of the genus *Lactarius* owe their colour changes to sesquiterpenoids. An example is *L. uvidus*, which has white latex which rapidly turns violet on exposure to the air. The compounds responsible for this colour change are mostly derivatives of sesquiterpene hydrocarbon drimane, (4a*R*,5*S*,6*S*,8a*S*)-1,1,4a,5,6-pentamethyltetrahydronaphthalene (**9-215**), such as drimenol (**9-216**), uvidin A and uvidin B (**9-217**) and their transformation products, respectively.



9-215, drimane

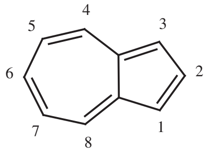


9-216, drimenol

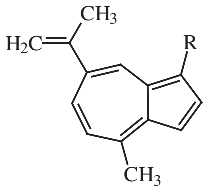


9-217, uvidin A, R = H
uvidin B, R = OH

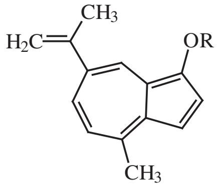
Blue pigments containing seven-membered rings are derived from sesquiterpene bicyclo[5.3.0]deca-1,3,4,6,8-pentaene, known as azulene (**9-218**). Azulene also gives the characteristic blue colour to chamomile oil (*Matricaria chamomilla*, Asteraceae). The pigments vetivazulene (4,8-dimethyl-2-isopropylazulene) and guaiazulene (1,4-dimethyl-7-isopropylazulene) are found in nature in mushrooms and some marine invertebrates. For example, the young fruiting body of *L. deliciosus* is first carrot-coloured, but slowly turns green on aging. The compounds responsible for these colour changes are the blue pigments 1,4-dimethyl-7-(1-methylethenyl)azulene, known as lactarazulene, and lipophilic red–violet 4-methyl-7-(1-methylethenyl)azulene-1-carbaldehyde, called lactaroviolin (**9-219**). However, neither lactarazulene nor lactaroviolin occur as such. The orange colour of the fungus is due to the labile and easily oxidisable dihydroazulen-1-ol (**9-220**, R = H) or to 1-hydroxymethyl-4-methyl-7-(1-methylethenyl)azulene stearate, R = $CO[CH_2]_{16}CH_3$. This lipophilic pigment is also responsible for the brilliant blue colour of the indigo milk cap (*L. indigo*) native to America and Asia, which has also been reported from southern France.



9-218, azulene



9-219, lactarazulene, R = CH_3
lactaroviolin, R = CH=O



9-220, dihydroazulen-1-ol (R = H)

9.12 Enzymatic browning reactions

Enzymatic browning reactions, which have been known for almost 100 years, are complex enzyme catalysed reactions involving the oxidation of phenolic compounds by some oxidoreductases in the presence of air oxygen. Oxidation products are quinones that are transformed by subsequent enzymatic and spontaneous (non-enzymatic) reactions into various coloured pigments. Quinones and other reaction products in plants have microbistatic effects and are able to prevent the spread of microbial and viral infections. Polymeric insoluble reaction products are an effective mechanical barrier against further erosion at the injury site of a plant.

In foods of plant and animal origin, the enzymatic browning reactions occur in damaged cells. The reaction is manifested by the formation of brown discoloration, carriers of which are melanin-type pigments. Enzymatic browning reactions in foods are usually adverse reactions in cases where they lead to browning and discoloration in food during processing and storage (browning of apples, potatoes or mushrooms). Adverse enzymatic browning reactions also occur in the processing of some seafood (e.g. shrimp and crabs). In some cases (such as in fermentation of tea, cocoa beans, ripening dates, production of black olives, raisins, sherry-type wines and aging of red wines), these reactions are to some extent desirable, because they lead to the formation of the characteristic colour and flavour of the product. Similarly to enzymatic browning reaction, autoxidation of phenolic compounds and their oxidation with oxidising agents, such as hydrogen peroxide also proceeds.

9.12.1 Enzymes

Enzymes from the class of oxidoreductases catalysing enzymatic browning reactions are most often known trivially as polyphenol oxidases. Two groups of enzymes can be identified:

- **catechol oxidases**
- **laccases**.

Catechol oxidases, formerly known as monophenols oxidases, catalyse two different reactions (Figure 9.31). The first reaction is oxidation (hydroxylation) of monophenols to *o*-diphenols (1,2-dihydroxybenzenes), which relates to the enzyme activity known as cresolase activity. These enzymes are now called monophenols oxygenases (an example is tyrosinase, which produces dopa from tyrosine). The second reaction catalysed by catechol oxidases is oxidation of *o*-diphenols to *o*-quinones (benzo-1,2-quinones also called 1,2-dioxobenzenes), which concerns the enzyme activity known as catecholase activity. Enzymes showing this activity were previously called diphenol oxidases or *o*-diphenolases and now are known under the systematic name *o*-diphenol:O_2 oxidoreductases. Some catechol oxidases lack this type of activity, but if they have this activity, then the ratio of creasolase and catecholase activities is usually 1 : 10 to 1 : 40. Laccases oxidise not only *o*-diphenols to *o*-quinones, but also *p*-diphenols to *p*-quinones (benzo-1,4-quinones also called 1,4-dioxobenzenes) (Figure 9.31). Other enzymes that are able to oxidise phenols to quinones are peroxidases. Their direct participation in enzymatic browning reactions is not yet fully understood.

Polyphenol oxidases are widespread in most plant species. They occur mainly in plastids of plant cells. For example, polyphenol oxidases in apples are located in chloroplasts and mitochondria, usually tightly bound to the membrane. The cytoplasm contains soluble polyphenoloxidases, whose activity increases with maturation.

The activities of polyphenol oxidases in plant tissues increase with mechanical injury. The activity depends mainly on the type, variety and age of plant material (e.g. unripe fruits turn brown faster), cultivation conditions and method of processing. The optimal pH for the enzyme activity is pH 4.5–5.5, but the enzymes are active even in more acidic media. For example, they show approximately 40% of optimal activity at pH 3. The highest activity of polyphenol oxidases is in the outer layers of fruits, especially in the skin. In animals, the enzyme tyrosinase is located mainly in the skin, hair and eyes.

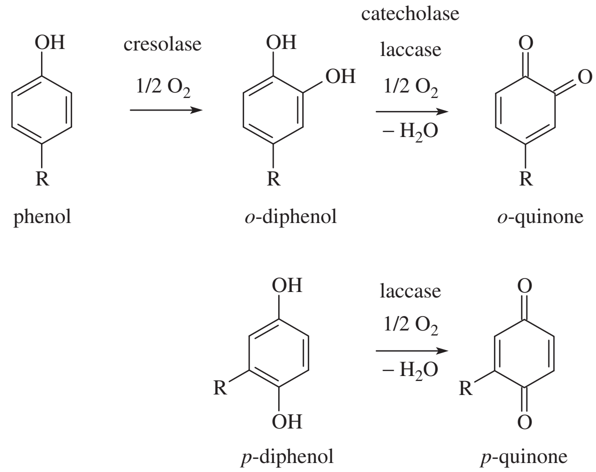


Figure 9.31 Reactions catalysed by polyphenol oxidases with creasolase, catecholase and laccase activities.

9.12.2 Substrates

The tendency of plant material towards browning varies within plant species, but the enzyme activity also depends on the type and amount of substrates available. The substrates of polyphenol oxidases may be a large number of different phenolic compounds, but usually only a few are of practical importance (Table 9.16). The individual compounds are given in the section dealing with sensorially active substances.

Phenolic compounds are present separately from the substrates, predominantly located in the cell vacuoles. Particularly prominent substrates are caffeic acid and its esters, as well as some flavonoid substances, of which monomeric flavan-3-ols (catechins) are the most important. Other groups of phenolic compounds, such as condensed forms of flavan-3-ols and flavan-3,4-diols (tannins), flavonols, flavones, flavans, chalcones, dihydrochalcones and anthocyanins, are only partly oxidised. One of the reasons for this is probably the steric hindrance caused by the corresponding glycosides and substrate specificity of polyphenol oxidases. The content of phenolic compounds depends on genetic factors (on plant species and varieties), the degree of maturity and external (environmental) factors (light, temperature, nutrients, use of pesticides and so on). The only substrate in animal tissues is the amino acid tyrosine.

9.12.2.1 Apples

The main substrates of polyphenol oxidases in apples are hydroxycinnamic acid derivatives, especially caffeic acid depsides, such as chlorogenic acid, and some groups of flavonoid compounds, such as flavan-3-ols, flavonols, dihydrochalcones and (in red-skinned apple varieties) also anthocyanins. In the flesh of apples, the main phenolics are chlorogenic (3-caffeoylquinic) acid, flavan-3-ol epicatechin, and procyanidin B_2 representing condensed tannins. These three compounds represent more than 90% of the phenolic compounds present. The main substrates in apple peel are flavan-3-ols and flavonols (quercetin glycosides) and to a lesser extent derivatives of hydroxycinnamic acids are present (chlorogenic acid and some other depsides).

The concentration of phenolic compounds is highest in young fruit and decreases rapidly with its development. After harvesting, the total content of phenolic compounds is roughly constant or slightly lower. The total content of phenolic compounds in ripe apples ranges between 0.5 and 11 g/kg in the flesh and between 8.7 and 19.2 g/kg in the skin. The phenolic compounds concentration in apple juice is around 1.5 g/kg.

Table 9.16 Important substrates of polyphenol oxidases in food materials.

Food	Substrate	Food	Substrate
Fruits		***Vegetables and potatoes***	
Apples	Chlorogenic acid, catechins, tannins	Lettuce	Tyrosine
Pears	Chlorogenic acid	Beans (green)	3,4-Dihydroxyphenylalanine (dopa)
Grapes	Catechins	Onion	Protocatechuic acid
Peches	Catechins	Potatoes	Tyrosine, chlorogenic acid, catechins
Olives	3,4-Dihydroxyphenylethanoland its derivatives	***Others***	
Bananasy	3,4-Dihydroxyphenylethylamine,	Mushrooms	Tyrosine, pulvinic acids and other phenols
Dates	Dactyliferic acid	Tea leaves	Catechins
Mango	Gallic acid, gallotannines	Coffee beans	Chlorogenic and caffeic acids
Avocado	Phenolic acids	Cocoa beans	Catechins

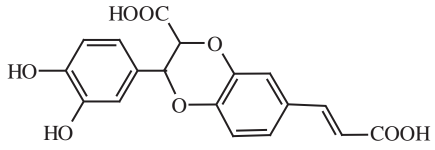
9.12.2.2 Potatoes

Common phenolics of potatoes are caffeic acid, its depsides (chlorogenic acid and its isomers), flavonol rutin and hydroxycinnamic acids and their conjugates. The content of phenolic compounds varies over a wide range depending on several factors, for example, variety. Chlorogenic acid and its isomers have been found in potato tubers in a range of from 0.3 to 13.7 g/kg dry weight, and rutin of from 0 to 190 mg/kg dry weight.

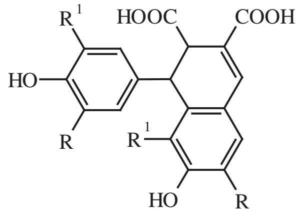
9.12.3 Non-enzymatic oxidation

In a neutral and alkaline medium, phenols spontaneously oxidise under the action of air oxygen, forming products of higher molecular weights. Some of these reactions are shown by the example of gallic acid in Figure 9.32. The main reaction product is hexahydroxybiphenylic acid, which is a biochemical precursor of ellagic acid. Hexahydroxybiphenylic acid and partly gallic acid also produce acyclic oxidation products.

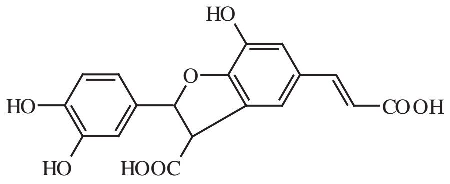
A large number of autoxidation products are similarly produced from cinnamic acids. The structures of certain dimers produced by autoxidation of caffeic acid in alkaline media at elevated temperatures are given in formulae **9-221**, **9-222** (R = OH, R^1 = H) and **9-223**. These dimers are lignans, trivially called **caffearins**. Products of similar structures arise from other cinnamic acids, for example the autoxidation product of sinapic acid is thomasidioic acid (**9-222**, R = R^1 = OCH_3). In neutral media of pH 7, thomasidioic acid is produced from about 30% of the initially present sinapic acid. In alkaline media of pH 8.5, virtually all sinapic acid is transformed into thomasidioic acid.



9-221, dimer of dioxolane type



9-222, dimer of cyclohexene type



9-223, dimer of furan type

Phenols can also be oxidised to the corresponding *o*-diphenols and *o*-quinones by hydrogen peroxide, which arises, for example, as a product of ascorbic acid oxidation, autoxidation of *o*-diphenols or autoxidation of cuprous ions in an acidic solution:

$$O_2 + 2Cu^+ + 2H^+ \rightarrow 2H_2O_2 + 2Cu^{2+}$$

In acidic fruit juices, hydrogen peroxide preferably oxidises other substances (e.g. in wine it oxidises ethanol to acetaldehyde and in strawberries it oxidises anthocyanins). Diphenols are relatively

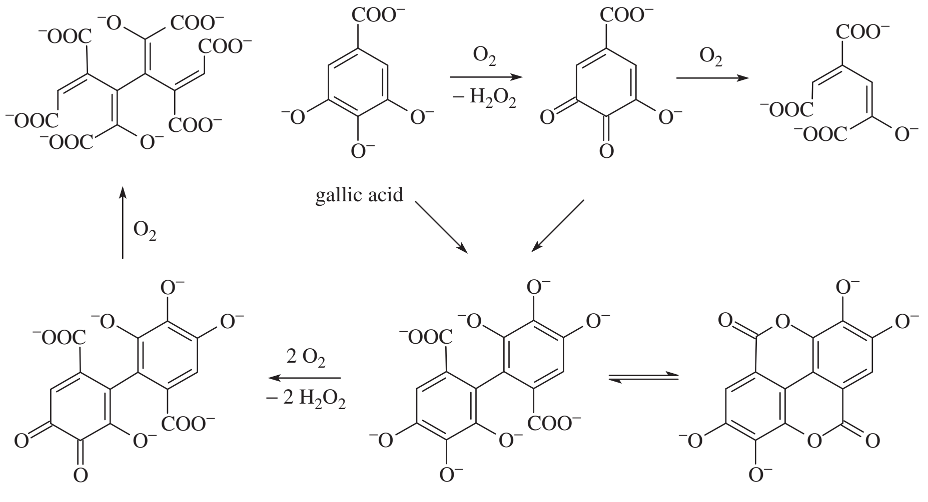


Figure 9.32 Gallic acid autoxidation in alkaline solutions.

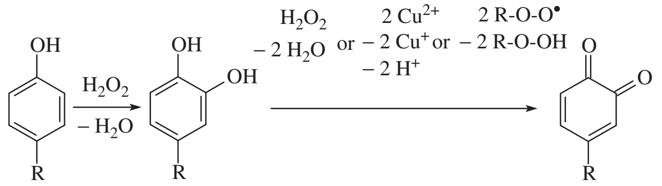


Figure 9.33 Non-enzymatic oxidation of phenols to *o*-diphenols and *o*-quinones.

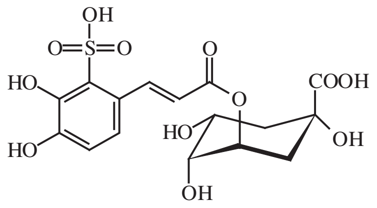
easily oxidised to the corresponding quinones by oxygen, metal ions (such as copper ions) or hydroperoxyl radicals (Figure 9.33), which arise in rancid fats.

9.12.4 Subsequent reactions

Quinones themselves are coloured compounds. Simple quinones are usually red when they contain an *o*-quinoid structure (such as *o*-benzoquinone) in the molecule, while compounds with the *p*-chinoid arrangement are yellow (e.g. *p*-benzoquinone). The catechin oxidation product is bright yellow, quinone formed of chlorogenic acid has a yellow–orange colour and quinone derived from amino acid dopa (dopaquinone) is pink.

o-Quinones formed by oxidation of phenolic compounds are highly reactive compounds. In food materials, they react with a variety of nucleophilic reagents to form adducts with the regenerated structure of the original diphenol, which have a lower redox potential than the original diphenol. These adducts are easily oxidised to the corresponding substituted *o*-quinones (Figure 9.34). The reactivity of *o*-quinones depends on the structure of the starting phenol, and is determined by the redox potential of reacting compounds. For example, *o*-quinones resulting from oxidation of chlorogenic acid may oxidise catechins to *o*-quinones and chlorogenic acid is regenerated, but the opposite reaction is not possible. Reactivity also depends on pH, temperature and the presence of other compounds. Reaction with water yields derivatives of 1,2,4-trihydroxybenzene as intermediates, which on oxidation produce hydroxy derivatives of *o*-quinones and *p*-quinones. Reactions of this type with tyrosine residues in the molecules of oxidoreductases lead to the inactivation of enzymes.

In materials containing ascorbic acid (abbreviated as H_2A), *o*-quinones are reduced to the original 1,2-diphenols of lighter colour and ascorbic acid is oxidised to dehydroascorbic acid (abbreviated A). This reaction can be monitored at home by the addition of lemon juice to tea, which results in a lighter tea colour. Quinones can be also reduced by hydrogen sulfites (bisulfites) or sulfites with the formation of the corresponding colourless 2′-sulfo derivatives (2′-sulfonic acids). The addition of hydrogen sulfites or sulfites to peeled potatoes is employed, for example, to prevent potato browning. The main 2′-sulfo derivatives identified in hydrogen sulfite-treated potatoes were derived from 5-*O*-caffeoyl-L-quinic acid (neochlorogenic acid, **9-224**) and 4-*O*-caffeoyl-L-quinic acid (cryptochlorogenic acid). The reaction with hydrogen sulfite includes the caffeic acid quinone produced by oxidation with polyphenol oxidase. The reaction is analogous to the reaction of quinones with amino acids (Figure 2.70). It proceeds via the nucleophilic attack of hydrogen sulfite anions to the C-2′ of quinones derived from chlorogenic acids, which yields 4′-anions of chlorogenic acids as intermediates. Other caffeoyl derivatives and derivatives of quercetin glycosides are produced as minor sulfo-adducts. Feruloyl and sinapoyl derivatives do not form



9-224, 2′-sulfo-5-*O*-caffeoyl-L-quinic acid

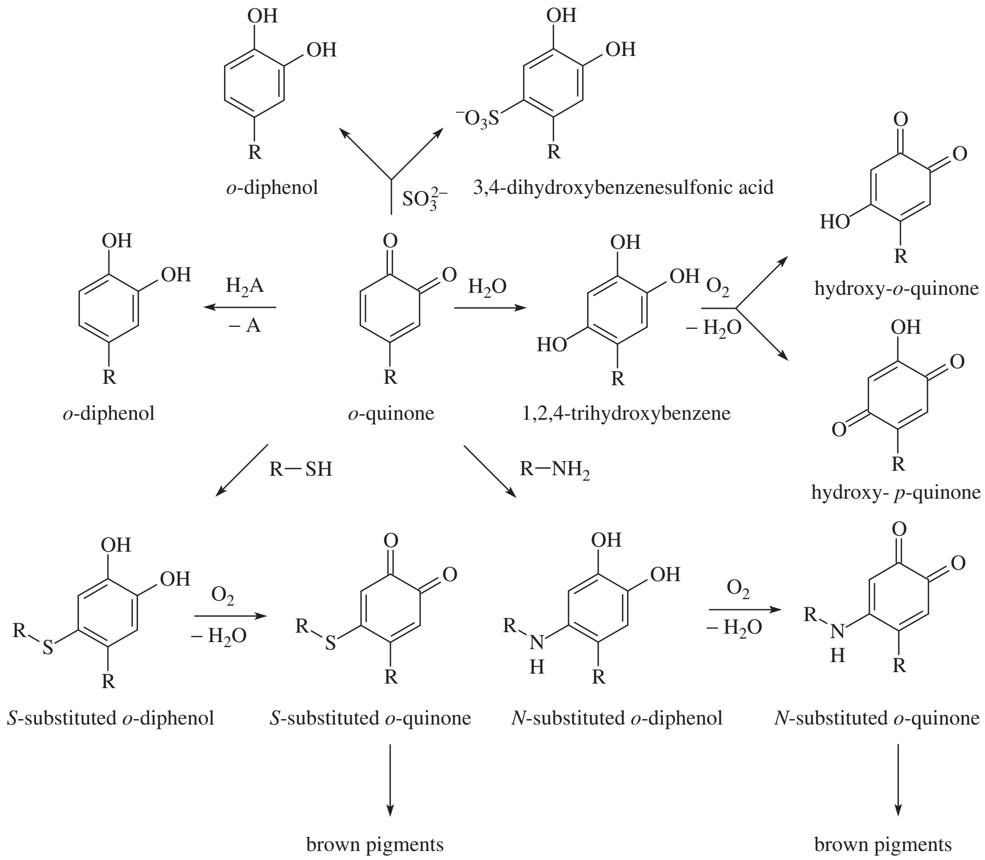


Figure 9.34 Reaction of quinones with food components.

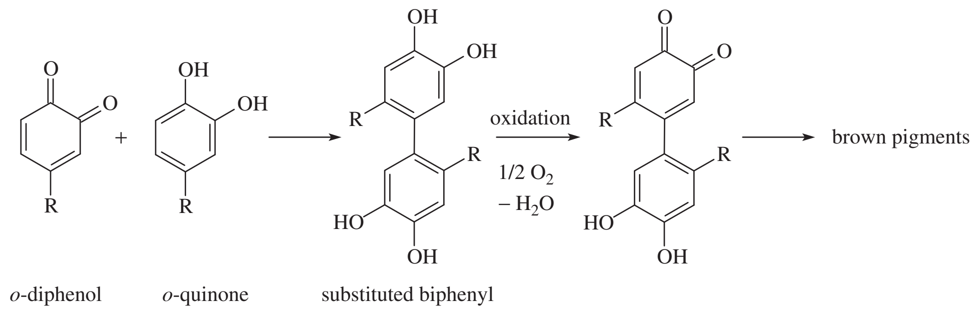
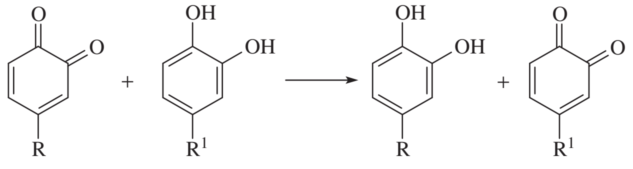


Figure 9.35 Condensation of quinones with *o*-diphenols.

adducts with hydrogen sulfites. Reactions leading to brown pigments are based on condensation of *o*-quinones with the original *o*-diphenols to dimeric products. A similar reaction also occurs, for example, in the formation of astringent tannins and pigments during red wine maturation. Dimers of phenolic compounds with the structure of *o*-diphenols are further oxidised by the action of polyphenol oxidases or by other quinones, and oxidation products condense again with phenols to give brown coloured higher oligomers and polymers (Figure 9.35). Quinones can even react with such *o*-diphenols, which are not otherwise oxidised to *o*-quinones. This reaction regenerates the original phenol, from which quinone was derived (Figure 9.36). Seven-membered tropone derivatives can also arise as reaction products. Their formation is analogous to the



Obrázek 9.36 Reaction of *o*-quinones with *o*-diphenols

Figure 9.36 Reaction of *o*-quinones with *o*-diphenols.

formation of theaflavin, which is produced during fermentation of green tea leaves.

Important reactions leading to the formation of brown polymeric pigments are reactions of quinones with thiol and amino groups

of proteins, which are described in detail in Section 2.5.2.5. In the presence of certain amino acids, quinones may yield stable low molecular weight coloured products and then the browning reaction cannot proceed. Some plant materials, such as apples, sweet potatoes (*Ipomoea batatas*, Convolvulaceae) and other raw materials, especially when treated with alkaline agents, such as baking powder, turn green during culinary processing. Greening can also be seen during extraction of sunflower meal protein with alkaline reagents. Greening cause caffeic acid esters, particularly chlorogenic acid, to be oxidised to the corresponding *o*-quinones that reacts with the primary amino group of amino acids or with primary amines. The mechanism of this green pigment formation is shown in Figure 9.37. The immediate precursor of the green pigment is yellow substituted trihydroxyacridine, the oxidation of which yields the green pigment. Reduction of the green pigment (e.g. with ascorbic acid) temporarily yields the yellow pigment. Oxidation of the green pigment in an alkaline solution produces a blue pigment. Amino acids with additional functional groups, such as serine and threonine, as well as proline and cysteine, do not form the green pigment.

9.12.4.1 Tea

The common commercial types of tea prepared from the leaves and buds of *Camellia sinensis* (Theaceae) are green tea, oolong tea and black tea (usually called red tea in China). The processing methods of the leaves are similar, except for the manner of fermentation and firing. The fermentation level increases from unfermented green tea to partially fermented oolong tea to well-fermented black tea. Firing involves heating at high temperature by means of a hot plate, hot air or flame after drying, and some species of green tea and oolong tea leaves are subjected to strong firing at >160 °C to enhance stability, taste and flavour.

Tea is one of the richest sources of flavonoids and phenolic acids. Green tea flavonoids are mainly catechins (flavan-3-ols) and their gallates, which constitute 80–90% of the phenolic compounds of leaves and 25–35% of their dry weight. The main catechins are epigallocatechin-3-*O*-gallate (7–13%), epigallocatechin (3–6%), epicatechin-3-*O*-gallate (3–6%), epicatechin (1–3%) and catechin (1–3% dry matter). Green tea also contains epicatechin and catechin oligomers, epicatechin-(4β→8)-epicatechin (procyanidin B_2), and catechin-(4α→8)-catechin (proanthocyanidin B_3) are also present, but their amount is lower than that of catechins. Important polyphenols are gallic acid and depsides of gallic acid with quinic acid, such as 1-*O*-galloylquinic acid (theogallin), 3-*O*-galloylquinic acid, 4-*O*-galloylquinic acid and 5-*O*-galloylquinic acid, and various di-*O*-galloyl, tri-*O*-galloyl, *O*-(digalloyl), *O*-galloyl-*O*-(digalloyl) and other depsides.

During fermentation of tea leaves, oxidoreductases oxidise catechins of green tea in enzymatic browning reactions, which leads to the formation of *o*-quinones that condense with catechins, forming

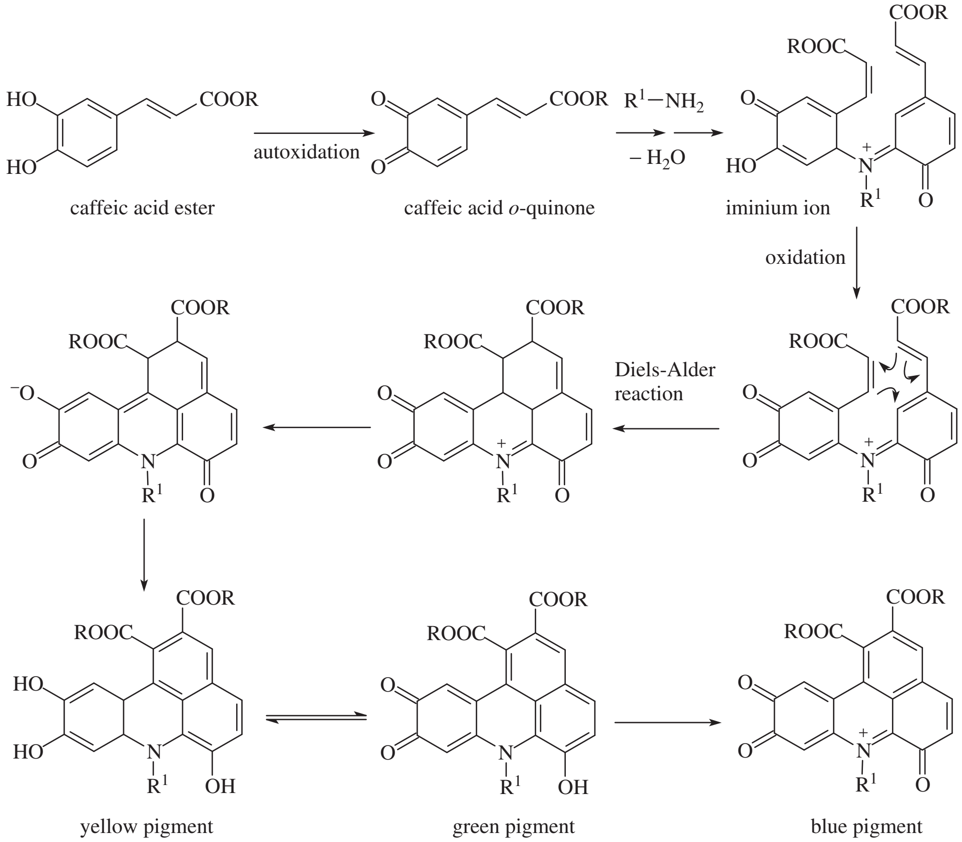


Figure 9.37 Formation of pigments from chlorogenic acid and amino acids.

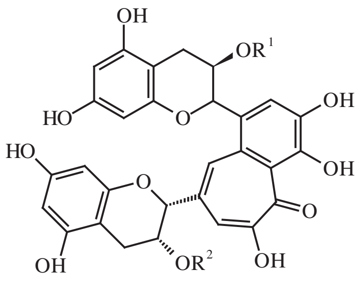
the coloured oolong or black tea pigments. Owing to differences in manufacturing, the types of polyphenols in green tea, oolong tea and black tea are very different. Traditionally two main groups of black tea pigments (collectively known as oxytheotannins) are recognised:

- **theaflavins**, soluble dimeric flavonoids of bright orange to red colour (about 10% of dry matter) containing a seven-membered tropone ring;

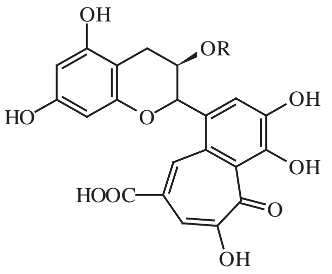
- **thearubigins**, very heterogeneous mixtures of red and yellow to orange–brown soluble to insoluble products of oxidation and polymerisation of catechins and gallates of unknown structure, having a relative molecular weight of 700–400 000 Da (about 23% of solids), low molecular weight compounds exhibit an astringent taste.

Apart from these two more or less defined groups of pigments, a number of other products of enzymatic reactions have also been identified in tea. Examples of these products are isotheaflavins, neotheaflavins, red epitheaflavic acid and its gallate, oxidation product of epigallocatechin-3-gallate called theasinensin A, colourless dimeric bisflavanols and dehydrotheasinensins, yellow theacitrins, polymeric brown theafulvins of not yet fully known structure and other products. About 20% of tea leaf flavonoids are transformed into insoluble compounds during fermentation. These are covalently bound to proteins via thiol groups and remain in the remnants of tea leaves in the preparation of brine. The dark colour of tea is also significantly influenced by phaeophytins arising from chlorophylls during fermentation.

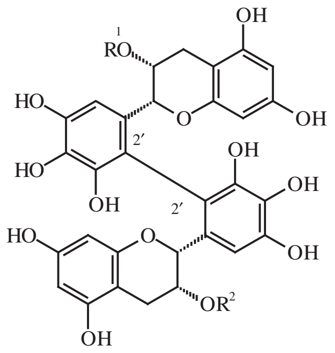
The structure of theaflavin, which is derived from epicatechin and epigallocatechin, is given in formula **9-225** as an example ($R^1 = R^2 = H$). Analogously, theaflavin-3-*O*-gallate ($R^1 = H$, $R^2 =$ galloyl) is produced from epicatechin and epigallocatechin-3-*O*-gallate, theaflavin-3′-*O*-gallate ($R^1 =$ galloyl, $R^2 = H$) from epicatechin-3-*O*-gallate and epigallocatechin and theaflavin-3,3′-*O*-digallate from epicatechin-3-*O*-gallate and epigallocatechin-3-*O*-gallate. The structure of epitheaflavic acid (R = H) derived from theaflavin is illustrated in formula **9-226**. Examples of products arising from epigallocatechin-3-*O*-gallate include 3-*O*-galloylepitheaflavic acid (**9-226**, R = galloyl), bisflavanols exemplified by bisflavanol A (**9-227**, $R^1 = R^2 =$ galloyl), theasinensin A (**9-228**), dehydrotheasinensins exemplified by dehydrotheasinensin A (**9-229**, R = galloyl) and theacitrins exemplified by theacitrin A (**9-230**, $R^1 = R^2 =$ galloyl).



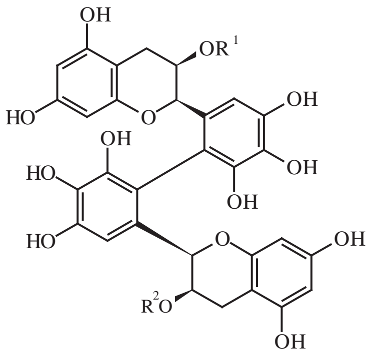
9-225, theaflavin ($R^1 = R^2 = H$)



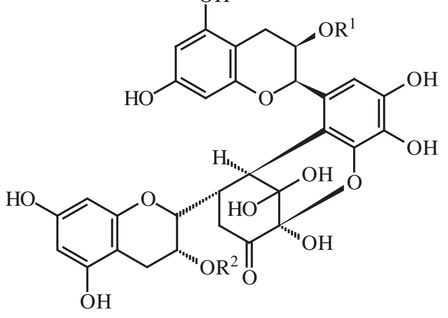
9-226, epitheaflavinic acid (R = H)
3-*O*-galloylepitheaflavinic acid (R = galloyl)



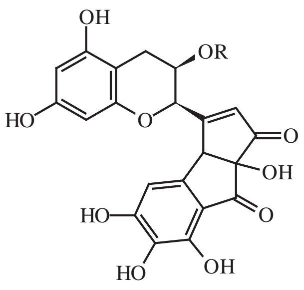
9-227, bisflavanol A ($R^1 = R^2 =$ galloyl)



9-228, theasinensin A ($R^1 = R^2 =$ galloyl)



9-229, dehydrotheasinensin A ($R^1 = R^2 =$ galloyl)



9-230, theacitrin A (R = galloyl)

9.12.5 Inhibition

The enzymatic browning reaction is an important technological problem, especially in the case of subsequent cold or freezing storage. Therefore, in addition to the reaction mechanisms, considerable attention is paid to its possible prevention. Prevention should begin early by selection of materials suitable for the given method of processing.

Fruits and vegetables with white flesh are prone to enzymatic browning, especially apples and pears, but also apricots, peaches, plums, potatoes and other materials. In most berries (raspberries, black- and redcurrants and blueberries) the enzymatic browning process has practically no impact due to the high content of natural pigments. The tendency to browning is due to activity of polyphenol oxidase and to the content and composition of phenols. Generally, it is possible to use the total content of phenols, tyrosine content or activity of polyphenol oxidase as criteria for selecting varieties for a particular type of treatment. One promising route may be breeding varieties with a lower tendency to enzymatic browning, or the use of gene manipulation. The technological inhibitory interventions can be divided into:

- physical methods
- chemical methods.

9.12.5.1 *Physical methods*

This group of methods includes gentle material handling before and during the process, where the intensity of browning, in this case induced by mechanical means, may be influenced by the style of some technological operations, such as clean cutting of the fruit.

The most important physical method of polyphenol oxidase inhibition is exposure of the material to higher temperatures. Polyphenol oxidases are inhibited by temperatures above 70 °C, are most stable in media of pH around 6.0, and in both directions from this value their resistance against heating decreases quite sharply.[5] Blanching, consisting in a rapid immersion of the material in hot water (salt or sugar solution) or exposure to water vapour, is used in the processing of vegetables that are eaten cooked and possibly also for the treatment of fruit pickles. Blanching is not very suitable for the inactivation of enzymes of fruits, which causes changes in consistency and organoleptic properties, nor is it suitable for the treatment of aromatic herbs used as spices.

Merely reducing the temperature reduces the reaction rate, but the colour changes are quite rapid even at 0 °C. This means that sensitive products that have not been pre-treated should be frozen as quickly as possible, for example mushrooms and sliced peaches.[6] Rapid intensive browning occurs during defrosting, when the activity of polyphenol oxidases increases due to disruption of cellular structures by ice crystals.

Another physical method of browning prevention is to restrict the access of oxygen. The production process for frozen sensitive materials uses four basic processes: packaging in hermetically sealed containers under either normal or reduced pressure; packaging in an inert atmosphere; and addition of sucrose. The first three methods differ only in the input treatment and their common feature is a temporary effect only for freezer storage. After opening the package at normal temperature, the browning reactions usually proceed even faster due to tissue disruption. The effects of low pressure can also lead to changes in fruit consistency. Dipping the fruit in sucrose solution before freezing or filling the entire package with sugar has, in addition to the suppression of browning, even more positive effects, especially increased viscosity of the liquid phase within the tissue. Significant inhibition of enzymatic reactions, however, occurs only at concentrations of sucrose higher than 20% as a result of increased osmotic pressure. Sugar also enhances the taste and aroma of fruit, but sweetening limits the possibility of further use of such processed raw material, which is then only suitable for the production of liqueurs, syrups, fruit purees and similar products.

Limiting access of air is also used in cold processing, such as in the production of so-called pre-processed vegetables and peeled cut potatoes. Using plastic foils with limited permeability for oxygen, foils impermeable to oxygen, or modification of the atmosphere can slow the browning reactions, but the composition of modified atmospheres must also take into account other physiological demands of packaged products.

9.12.5.2 *Chemical methods*

Another option to reduce or inhibit the enzymatic browning reaction is the use of chemical reagents. Many substances capable of inhibiting polyphenol oxidases are known, but the mechanism of their action is often unknown. Complexing agents are able to bind copper ions, which the enzyme requires. Interaction with

[5]The enzyme sensitivity towards heating is expressed by thermoinactivation curve empirically obtained as a dependence of temperature on the logarithm of heating time, after which the enzyme activity is reduced by one order. The tangent line to the curve for polyphenol oxidase = 12 °C. For inactivation, heating at a temperature of 82 °C for 1.1 min (that means $F_{82} = 1.1$ min) is sufficient.

[6]Effect of temperature on reaction rate is expressed by the coefficient Q_{10}, which is equal to the ratio of rate (e.g. rate constant) at temperature $t + 10$ °C and the rate at temperature t. Coefficient Q_{10} for enzymatic browning reactions has a relatively high value (e.g. for the browning of peaches $Q_{10} = 18$). Therefore, browning of peaches does not occur during freezer storage (−18 °C) thus stored fruits are sufficiently stable.

Table 9.17 Inhibitors of enzymatic browning reactions.

Compound	Mechanism of action
Tropolone (2-hydroxycyclohepta-2,4,6-trien-1-one)	Copper binding
Citric acid	Copper binding, acidification
Phosphoric acid, polyphosphates	Copper binding, acidification
Ethylenediamintetraacetic acid (EDTA)	Copper binding, slight acidification
D-Gluconic acid kyselina	Copper binding, slight acidification
Sodium chloride, sodium fluoride	Copper binding
Benzoic, *p*-coumaric, cinnamic and feruic acids	Competitive enzyme inhibition
4-Hexylresorcinol and other 4-substituted resorcinols	Competitive enzyme inhibition
Proteases (ficin from figs, actinidin from kiwi, papain from papaya, bromelain from ananas)	Enzyme inhibition
Sodium hypochlorite, calcium hypochlorite	Enzyme inhibition
Modified substrates, such as (+)-catechin 3′-*O*-α-D-glucoside	Enzyme inhibition
Cyclodextrins	Formation of complexes with phenols
Ascorbic acid and its derivatives	Reduction of quinones, slight acidification
Hydrogensulfites, sulfur dioxide	Reduction of quinones and addition products, enzyme inhibition
Thiols (cysteine, *N*-acetylcysteine, glutathion)	Reduction of quinones and addition products, enzyme inhibition

copper is also the cause of the inhibitory effects of some inorganic ions, especially halides. Substances structurally similar to substrate (such as benzoic and cinnamic acids and their derivatives) may competitively inhibit polyphenol oxidases. Also considered is the possibility of direct influence on the active sites of enzymes by free radicals, which can be produced, for example, from added ascorbic acid. The inhibitory effect of hydrogen peroxide on mushroom tyrosinase is explained by the same mechanism. Acidification below the polyphenol oxidase optimum pH (pH 4–4.5) may also slow or stop the browning reactions.

Another group of inhibitors of polyphenol oxidases are reducing agents capable of reducing the resulting quinones back to diphenols (ascorbic acid, thiols and some other agents). The antibrowning effect of ascorbic acid has been associated with its ability to reduce quinones to their precursor phenolics and with lowering the pH with a concomitant inhibition of polyphenol oxidase activity. However, if the effect of the inhibitor is a mere reduction of quinones, browning is inhibited only until the exhaustion of the reagents. The sulfur-containing agents (such as bisulfites) seem to control the browning reaction by irreversible inactivation of polyphenol oxidase as well as by reacting with quinones to produce colorless compounds (see Section 9.12.4). An overview of the main inhibitors is given in Table 9.17.

The practical use of chemical inhibitors is limited by several factors. Most substances with a strong inhibitory effect, when tested in model systems, are not effective in real materials, where other factors also play a role. It is easier to use an inhibitor in a homogeneous material, such as juice or puree. In the case of cut fruits, leaves and spices there are two possible alternatives: soaking the material for some time in the inhibitor solution or spraying the inhibitor over the material surface. Individual substances differ in their ability to penetrate the plant tissue. Bisulfites in particular are very effective. Another limitation may be material properties. For example, the use of acid is avoided in green plants, where browning will be suppressed, but the acid will accelerate the conversion of chlorophylls to phaeophytins (phaeophytinisation).

Health regulations normally allow only the use of ascorbic acid and some of its derivatives, citric acid, sodium chloride and hydrogen bisulfites. Hydrogen bisulfites and sulfur dioxide are very effective in protection against browning and are widely applied (e.g. in packed peeled potatoes). In practical terms, mixtures of citric and ascorbic acids are used. As a very effective means to stabilise the colour of apple slices and other fruits, a mixture of phosphates, citric acid and glucose is recommended. The use of an inhibitor is often part of a complex procedure also involving other methods, especially the limitation of air access. No agent (perhaps with the exception of hydrogen sulfites) and no treatment are universally applicable. A prerequisite for a reliable method of prevention and inhibition of browning is the search for perfect ways of treating individual materials appropriate to the available technology and processing methods.

10

Antinutritional, Toxic and other Bioactive Compounds

10.1 Introduction

As we have seen in the preceding chapters, foods contain many beneficial and also indifferent substances. In this chapter, we shall discover that foods can also contain antinutritional and toxic substances. Antinutritional substances found in the human diet or animal feed have the potential to adversely affect health, as they may interfere with the body's ability to digest and utilise nutrients, especially proteins, vitamins and minerals. It has been said that about 99% of all toxins are naturally occurring, and also that all things are toxic at a sufficiently high concentration. Certainly, many food raw materials contain chemicals, which, if consumed in excess, might lead to health problems. Terminology and definitions for materials that cause toxic effects are not always used consistently. A toxic substance or toxicant is a substance that produces an adverse biological effect of any nature, and which may, depending on the extent of exposure (dose), cause disease or damage to the body. Toxicity is the ability of a substance to produce an unwanted effect when the compound has reached a sufficient concentration at a certain site in the body. Toxicants causing immediate toxic effects (death or illness) when experienced in very small amounts are known as poisons. Natural toxic substances, mainly specific proteins produced by living organisms, such as microbial and mushroom toxic products, are called toxins. Toxinogenicity is the ability of organisms to produce toxins.

Depending on the origin, antinutritional and toxic substances include two types of compounds:

- natural components that are natural antinutritional and toxic compounds, found in food as a result of genetic predisposition of organisms;
- foreign substances.

Foreign substances are divided according to the way they get into food:

- compounds added intentionally to improve the food quality during processing, storage and other operations (extending their shelf life, improving sensory, nutritional and technological quality) that are therefore classified as additive substances or **food additives** (see Chapter 11);
- compounds getting into food accidentally from the environment during production, storage, transportation, marketing and other manipulations or arising in physical and chemical processes of food production that are classified as **pollutants** or **food contaminants**; a special case is food contamination by microbial toxins (bacterial toxins and mycotoxins) (see Chapter 12).

Products of anthropogenic activities that do not occur in nature are called **xenobiotics** (from the Greek word *xenos* meaning foreign). In relation to food, we often distinguish exogenous or primary contaminants (contaminants from the environment, such as residues of pesticides or residues of veterinary medicines used in agriculture) and endogenous or secondary contaminants that arise during food processing from natural food components by different physical and chemical factors (such as products of ionising radiation, thermal and oxidation reactions). According to some classifications, food additives are not regarded as foreign substances, but natural toxic substances in food are regarded as food contaminants. The nature, properties, adverse effects and analysis of toxic substances related to food and the human food chain with respect to the human organism and disease manifestations in humans are of interest in food toxicology.

Natural toxic substances and food contaminants may exhibit different effects (biological effects) upon living organisms, largely depending on where and how they are taken in:

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- orally (through the mouth)
- by inhalation (through breathing)
- by absorption through mucous membranes and skin (dermally).

Adverse biological effects, bringing the risk of possible poisoning are called toxic effects. Toxic effects may manifest as:

- **acute**, after a single dose of a toxin[1]
- **chronic**, after repeated or continuous administration of toxic substances (over a period of time).

On the basis of biological action, toxic substances may, for example, act as:

- **asphyxiants** (exerting their effects through a depletion of oxygen to the tissues – asphyxia, such as carbon monoxide and hydrogen cyanide);
- **carcinogenic agents** or carcinogens[2] (inducing cancer, such as some lipophilic synthetic dyes, acrylamide, polycyclic aromatic hydrocarbons, *N*-nitrosamines, polychlorinated dibenzodioxins and dibenzofurans);
- **hepatotoxic agents** or hepatotoxins (producing liver damage, such as pyrrolizidine alkaloids, aflatoxins, amatoxins and phallotoxins);
- **irritants** and **sensitising agents** (irritation occurs from contact with irritants, such as ammonia, allyl isothiocyanate and some essential oils of spices; sensitisation is a reaction after exposure to sensitising agents that may cause allergic responses, such as hypericin, fagopyrin and some coumarins);
- **mutagenic agents** or mutagens (that may cause cancer or undesirable mutations, such as reactive oxygen species, some carbonyl compounds and aromatic amines);
- **nephrotoxic agents** or nephrotoxins (producing kidney damage, such as some halogenated hydrocarbons, amatoxins and phallotoxins);
- **neurotoxic agents** or neurotoxins (that may damage nervous tissue), such as 3-(*N*-oxalyl)-L-2,3-diaminopropionic and ibotenic acids;
- **teratogenic agents** or teratogens (that may cause defects in the foetus in pregnant women (such as alkaloids chaconine, tomatine, anabasine and anatabine).

According to the potential dose-related risk, the following categories are usually recognised:

- **contaminants** (mainly microbial toxins, as well as industrial contaminants, residues of veterinary drugs and pesticides), which can be a significant risk, but the long-term effects are difficult to estimate;
- **natural toxicants**, where risks exist, but knowledge of the effects is generally inadequate;
- **additives**, where the risks are small (with a few exceptions, which are nitrites and some flavouring substances);
- **antinutritional substances**, where risks are potential rather than real, which is largely derived of antinutritional effects manifested in animals.

The maximum amounts of antinutritional substances, natural toxins, additives and contaminants which may be present in wholesome foods and tobacco products are indicated in the relevant legislation, as a result of toxicological and epidemiological studies (using data on the food consumption). These data include safety factors so that foods containing toxic substances can be consumed daily over a lifetime without demonstrable negative effects.

To date, more than a dozen terms or measures are used to denote directly or indirectly a health risk as significant or insignificant. These risk measures include, but are not limited to: NOEL, NOAEL, RfD and ADI. In animal experiments (at least in two animal species, although the intention is to replace these experiments *in vivo* by testing *in vitro* using cell or tissue cultures), the ineffective concentration of a toxic substance that does not result in observable damage to the health of the animal is determined. This ineffective concentration (a threshold in mg per kg body weight, below which adverse effects do not occur) of a substance is called the No Adverse Effect Level (NOAEL). Toxicologists can also report results as the Lowest Observed Adverse Effect Level (LOAEL). These numbers, preferably the NOAEL and sometimes the LOAEL values, are used by risk assessors and regulatory agencies to determine such values as the Reference Dose (RfD) for a substance used by the USEPA (U.S. Environmental Protection Agency). RfD is the dose or concentration that is assumed to cause no harm to human populations upon daily exposure to that substance. $RfD = NOAEL$ (or LOAEL)/$UF \times MF$, where UF is uncertainty factor, which is often a tenfold factor for each uncertainty, and MF is modifying factor, which can range between 0 and 10, with 1 as the default; UF is based on animal tests and animal to target species extrapolation, along with other causes of uncertainty.

The term ADI (Acceptable Daily Intake, typically expressed in mg/day per kg body weight) was first used by the joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives and is now used extensively by the USFDA (U.S. Food and Drug Administration). ADI is the predetermined

[1]The acute toxicity of a compound is generally measured by experiments on animals (quite often rats). The most common measure of acute toxicity is lethal dose value (LD_{50}) usually given in weight of compound per kg of body weight (also used to classify poisons), which causes death in 50% of the test animals.

[2]The International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO), recognises the following groups of carcinogenic substances: Group 1 – carcinogenic to humans, Group 2A – probably carcinogenic to humans, Group 2B – possibly carcinogenic to humans, Group 3 – unclassifiable as to carcinogenicity in humans and Group 4 – probably not carcinogenic to humans.

amount of a substance (such as a pesticide or food additive) that can be consumed daily by humans for an entire lifetime without causing appreciable adverse effects. ADI is calculated according to the equation: ADI = NOAEL/SF, where SF is safety factor, usually of a multiple of 10, to account for intraspecies sensitivity, interspecies difference and other factors of concern (such as completeness of data). If it concerns a toxicologically significant effect of a toxic substance (such as reproductive disorders) or if sufficient data are not available, the SF value is increased to 100 or even to 1000.

In the case of pesticides, the calculation of Maximum Residue Levels (in mg/kg food) also reflects the quantity of food consumed. The MRL is calculated from the equation: MRL = ADI × body weight/food consumption in kg. If the same toxic substance is present in several foods, then the food basket (food per day) is examined, and the total amount of toxic substance must not exceed the determined value. Information about the chronic effects of toxic substances is provided by long-term tests on sub-chronic and chronic toxicity.

Toxicological evaluation of veterinary drug residues in food is based on the toxicological and pharmacological effects of antimicrobial veterinary drugs (antibiotics) on representative pathogenic and non-pathogenic microflora of the human digestive tract and representative microorganisms of the genera *Lactococcus*, *Leuconostoc*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Propionibacterium* used in biotechnology. The equation used for deriving the ADI value (in μg/kg body weight) is calculated using the following equation $ADI = MIC_{50} \times MCC/FA \times SF \times BW$, where MIC_{50} = Minimal Inhibitors Concentration (the minimum inhibitory concentration in μg/g for 50% of test organisms), MCC = Mass of Colonic Content (amount of 220 g is used), FA = Fraction of an oral dose Available to act upon microorganisms in the colon, SF = Safety Factor (ranging from 1 to 10, a value of 1 being used when extensive, relevant microbiological data are available) and BW = body weight (60 kg).

According to current knowledge, however, a number of natural compounds formerly classified as toxins or indifferent substances show desirable and even beneficial biological effects. The term bioactive food component refers to non-essential molecules that are present in foods and exhibit the capacity to modulate one or more metabolic processes, which results in the promotion of better health. Bioactive food components also have multiple metabolic activities allowing for beneficial effects in several diseases. In general, it is thought that bioactive food components are predominantly found in plant foods, such as cereals, nuts, fruits and vegetables. For example, isothiocyanates and other degradation products of glucosinolates, occurring in broccoli, cauliflower, Brussels sprouts and other *Brassica* vegetables, were known as potential antithyroid compounds, but it has been shown that many of them induce detoxifying enzyme systems and have antimicrobial, immunomodulatory and anticancer properties. Phytooestrogens of soybeans, soy-based products and flaxseeds have anti-oestrogenic, anti-osteoporotic and anti-proliferative properties. While a few plant saponins are toxic in large doses, most saponins found in food (cereals, legumes, potatoes, spinach, tomatoes and other products) are safe and may even have many beneficial health effects. Studies have illustrated the beneficial effects of some saponins on blood cholesterol levels (by forming complexes with cholesterol and bile acids), a hypolipidaemic effect, or show antioxidant, antimutagenic and anticarcinogenic properties and stimulate the immune system,[3]

Attention in this chapter is paid to natural antinutritional and toxic substances of food and to those raw materials that are used in the diet directly or after technological and culinary processing. Attention is also paid to natural substances that enter the food chain indirectly through livestock feed. The first part of this chapter deals with antinutritional compounds (enzyme inhibitors, antivitamins, compounds binding minerals and other antinutritional factors). Toxic food constituents are divided into two large groups: compounds that are toxic for certain individuals and cause food intolerance (such as non-allergic food hypersensitivity and immune system reactions known as food allergies) and compounds that are toxic for the whole population. The second group of toxic constituents of food includes alkaloids found in edible plants and plants that are consumed by livestock, saponins, cyanogens (cyanogenic glycosides and lipids), and glucosinolates. Important phenolic compounds (oestrogens and phototoxic compounds), lectins, toxic amino acids, biogenic amines and polyamines and, last but not least, marine toxins (shellfish and fish toxins) and toxic substances of mushrooms. Each section concentrates on the structure and occurrence of antinutritional and toxic compounds, and their biological effects, changes and reactions in raw materials and foods. However, a number of the so-called toxic substances and their decomposition products are beneficial at low concentrations for human well-being and health, and many find use in the pharmaceutical industry and medicine.

10.2 Antinutritional compounds

Antinutritional substances include:

- enzyme inhibitors (antienzymes)

[3]Most foreign substances (food contaminants, xenobiotics, but also natural toxins) are normally metabolised in two phases. The first phase is mainly oxidation catalysed by microsomal enzyme systems. In the second phase, the oxidised metabolites are converted into polar compounds (e.g. in conjugation reactions with D-glucuronic acid or glutathione) and are eliminated from the body. During the first phase, however, some compounds (called procarcinogens, which are carcinogens requiring metabolic activation) may produce highly reactive metabolites able to attack the DNA and initiate the development of cancer. Development of cancer is a multi-step process that can be arbitrarily subdivided into initiation, promotion and progression steps.

Every organism has, however, protective systems, which may hinder the process of carcinogenesis. Substances with anticarcinogenic activity can be divided into three groups according to the mechanism of action. The group A anticarcinogens inhibit enzymes involved in the metabolic activation of procarcinogens, but are ineffective for carcinogens, which do not require activation (so-called direct carcinogens). The other two groups are substances that efficiently convert carcinogens into harmless products. Group B consists of substances that activate the detoxification enzyme systems (such as glutathione-*S*-transferase, which catalyses the binding of a variety electrophiles to glutathione thiol group). Group C substances reduce the risk of DNA damage, either by directly reaction with electrophiles or by preventing their reaction with DNA. Naturally occurring anticarcinogenic compounds can play an important role in cancer prevention by inhibiting one or more of the important steps in tumour cell development.

- antivitamins
- compounds interfering with the metabolism of minerals
- antinutritional phenolic compounds
- antinutritional oligosaccharides.

10.2.1 Enzyme inhibitors

Enzyme inhibitors, also known as antienzymes, are various low molecular weight and high molecular weight natural food components and foreign substances that affect the activity of various enzymes. In nutrition terms, the most important antienzymes are inhibitors of digestive enzymes, particularly **inhibitors of proteases** also known as antiproteases. A less important group are **inhibitors of saccharases**.

10.2.1.1 Inhibitors of proteases

Protease inhibitors, which are important from the nutritional point of view, are proteins or polypeptides capable of inhibiting the digestive enzymes proteases.

10.2.1.1.1 Classification

Protease inhibitors are classified according to the type of proteases, which are inhibited. Recognised inhibitors are:

- serine protease inhibitors (e.g. inhibitors of trypsin, chymotrypsin, elastase and plasmin)
- sulfhydryl protease inhibitors (e.g. inhibitors of pepsin and thrombin)
- acidic protease inhibitors (e.g. inhibitors of cathepsin D)
- metaloprotease inhibitors (e.g. inhibitors of pancreatic carboxypeptidase).

The most important inhibitors are inhibitors of serine proteases, which include two main groups of protease inhibitors:

- Kunitz type inhibitors (KI)
- Bowman–Birk type inhibitors (BBI).

10.2.1.1.2 Occurrence and properties

Protease inhibitors are naturally present in plant foods, especially in legume seeds, but they also occur in cereals and some other plant materials (such as potatoes and tomatoes). Protease inhibitors of microbial and animal origins also exist. In plants, protease inhibitors fulfill several functions. They probably serve as a cytosol protection against endogenous proteases released in disrupted cellular structures. In addition, they act as storage proteins during seed germination and are involved in the protection of plant tissues against elicitors (viruses, bacteria and fungi) and predators (animals).

Kunitz type inhibitors

Kunitz type inhibitors have a relative molecular weight of about 20 kDa and have two disulfide bridges in the molecule. They primarily exhibit specificity to trypsin. Examples are Kunitz type inhibitors of soya beans called STI (Soybean Trypsin Inhibitor), which are a group of isoinhibitors with relative molecular weights of 18–24 kDa. The main component is a protein containing 181 amino acid residues and two disulfide bridges (20 kDa). The binding sites, where the inhibitor interacts with trypsin, are the amino acid residues Arg^{63} and Ile^{64}. A complex with trypsin arises in a stoichiometric ratio (one inhibitor molecule interacts with one molecule of trypsin). The complex is analogous to the enzyme–substrate complex, but does not in practice dissociate into the original protein (trypsin) and enzyme inhibitor.

Bowman–Birk type inhibitors

Inhibitors of the Bowman–Birk type have a relative molecular weight of about 6–10 kDa, a higher number of disulfide bridges, and exhibit specificity against trypsin and chymotrypsin, as they contain two independent binding sites in the molecule. Bowman–Birk type inhibitors belong to the most common inhibitors. They occur in legumes, cereals, pseudocereals, potatoes and some other materials.

In soya beans (*Glycine max*, Fabaceae), for example, five isoinhibitors of this type occur, which belong to the group of PI (Potato Inhibitor) and are labelled PI-I to PI-V. In potatoes, as well as in barley, inhibitors PI-I and PI-II occur. Inhibitor PI-I is a polypeptide of molecular weight of 8 kDa, which contains a polypeptide chain consisting of 71 amino acids and seven disulfide bridges. The inhibitor PI-I has dual binding specificity. The binding sites of trypsin are amino acids Lys^{16}-Ser^{17}, while the interaction with chymotrypsin occurs via amino acids Leu^{44}-Ser^{45}. Similar structures and properties have inhibitors present in other legumes and oilseeds. Common bean (*Phaseolus vulgaris*) contains inhibitor GBI (Garden Bean Inhibitor), the lima (butter) bean (*P. lunatus*) contains inhibitor LBI (Lima Bean Inhibitor), in chickpeas (*Cicer arietinum*) is found inhibitor CPI (Cow Pea Inhibitor) and in peanuts (*Arachis hypogaea*) inhibitor GI (Groundnut Inhibitor).

Buckwheat (*Fagopyrum esculentum*, Polygonaceae) contains the inhibitor BTI (Buckwheat Trypsin Inhibitor), and, correspondingly, three isoinhibitors of the Bowman–Birk type that are called BTI-1, BTI-2 and BTI-3, which consist of a single polypeptide chain containing 69 amino acids. The content of protease inhibitors in selected legumes is shown in Table 10.1.

Sulfhydryl protease inhibitors, for example, are found in the seeds of legumes of the genus *Vigna*; inhibitors of acid proteases occur in potatoes, and inhibitors of metaloproteases occur in potatoes, tomatoes and other crops.

Table 10.1 Protease inhibitors content in selected legumes.

Legume seed	Content of protease inhibitors (g/kg)	
	Kunitz type inhibitors	Bowman-Birk type inhibitors
Soya bean (*Glycine max*)	20	2–3
Peas (*Pisum sativum*)	–	0.05–3
Cowpea (*Cicer arietinum*)	–	2.3

10.2.1.1.3 Mechanism of action

Knowledge of the antinutritive effects of protease inhibitors in humans is still limited and mainly derived from the knowledge gained in animal nutrition. When feeding raw or undercooked legumes to farm animals, inhibitors of proteases (trypsin inhibitors) cause disturbances that are manifested by slow growth of the animals. In chronic cases it leads to enlargement of the pancreas, which is histologically described as hypertrophy (enlargement of the pancreas or its parts due to enlargement of individual cells) and hyperplasia (enlargement of pancreas by pancreatic cell multiplication). This is due to increased secretion of digestive enzymes, including trypsin, chymotrypsin and elastase by the hyperactive pancreas. It is thought that the growth retardation of animals is actually the result of endogenous loss of amino acids, particularly sulfur amino acids, which are used for the synthesis of proteases that subsequently pass into the faeces and cannot be used for the synthesis of muscle proteins.

Trypsin inhibitors are not the only antinutritive factors in soya beans that may slow the growth of farm animals. For example, the slowdown of animal growth, for which trypsin inhibitors are responsible, is about 40% of the total antinutritive activity of raw soya beans. About 25% of the antinutritive activity can be attributed to lectins and the rest to other antinutritional substances.

10.2.1.1.4 Inactivation

Adverse effects of protease inhibitors are relatively easy to eliminate by thermal processing of plant materials. Reduction of inhibitor activity depends on temperature, time of heating, material particle size and water content. For soya beans, the most common way of thermal inactivation of protease inhibitors is the so-called toasting process (effect of water vapour), but other methods are also effective, such as boiling in water, dry roasting, microwave heating and extrusion. For example, trypsin inhibitors of most commercial soya products for human consumption (tofu, soya beverage, incorrectly called soya milk, soya isolates and concentrates and textured soya meat substitutes) are sufficiently inhibited showing only about 20% of the activity of raw soya beans. Inactivation of inhibitors to 50–60% is necessary to eliminate their adverse effects (animal growth retardation and impaired pancreatic function). Germination of mature soya beans also gradually decreases the activity of Kunitz type inhibitors, because modified forms of these proteins arise by proteolysis and *de novo* synthesis.

10.2.1.2 Inhibitors of saccharases

Many cereals and cereal products (such as wheat, rye, breakfast cereals and bread) contain proteins inhibiting animal, but not plant amylases. Invertase inhibitors are found in potato tubers, for example. The significance of these enzyme inhibitors is negligible and the consequences of their presence in food are not yet known. It is believed that amylase inhibitors could potentially be used in preparations for diets to promote weight loss.

10.2.2 Antivitamins

Antivitamins (vitamin antagonists) are those substances that eliminate the biological effects of vitamins, which can lead to deficiency symptoms. Antivitamins are dealt with in detail in Chapter 5.

10.2.3 Compounds binding minerals

In foods of plant origin, certain compounds are found as natural components that interfere with the metabolism of minerals. The most important groups of these compounds include:

- **phytic acid** and **phytin**
- **oxalic acid**
- **glucosinolates** and their degradation products.

10.2.3.1 Phytic acid and phytin

Phytic acid, *myo*-inositol-1,2,3,4,5,6-hexakisdihydrogenphosphate, is the main storage form of phosphorus used during germination of seeds of cereals, pulses and oilseeds. Phytic acid in seeds occurs primarily as a mixed calcium and magnesium salt, which is called phytin. Insoluble salts and ones that are utilised only slightly are also produced with other di- and trivalent ions, such as Fe and Zn in particular. The issue of phytin is discussed in detail in Chapter 6 (see Section 6.3.4.2.1).

10.2.3.2 Oxalic acid

Oxalic acid is a common component in many vegetables and other plant foods (Table 8.15). Oxalic acid yields insoluble calcium oxalate

with calcium ions, which may under certain circumstances (existing low intake of calcium and vitamin D) seriously interfere with the metabolism of calcium. It also occurs in the so-called beer stone, a greyish brown scale composed of calcium oxalate and organic substances, which forms on the inside surfaces of brewing apparatus.

Approximately 75% of all human kidney stones are composed primarily of calcium oxalate and hyperoxaluria, also known as oxalosis (urinary oxalate excretion that exceeds 40 mg/day) is a primary risk factor for this disorder. Urinary oxalate originates from a combination of absorbed dietary oxalate and endogenously synthesised oxalate, nevertheless boiling of vegetables markedly reduces dietary soluble oxalate content by 30–87% and is more effective than steaming (5–53%). The loss of insoluble oxalate during cooking varies greatly, ranging from 0 to 74%. Because soluble sources of oxalate appear to be better absorbed than insoluble sources, employing cooking methods that significantly reduce soluble oxalate may be an effective strategy for decreasing oxaluria in individuals predisposed to the development of kidney stones.

10.2.3.3 Glucosinolates

Glucosinolates, especially the glucosinolate progoitrin (whose decomposition yields the antithyroid substance goitrin), but also some other glucosinolates which produce antithyroid isothiocyanates (such as sinigrin, which produces allyl isothiocyanate) or antithyroid thiocyanates (such as indole glucosinolates) are often classified as compounds that interfere with the metabolism of iodine. The issue of glucosinolates is given elsewhere (see Section 10.3.2.4).

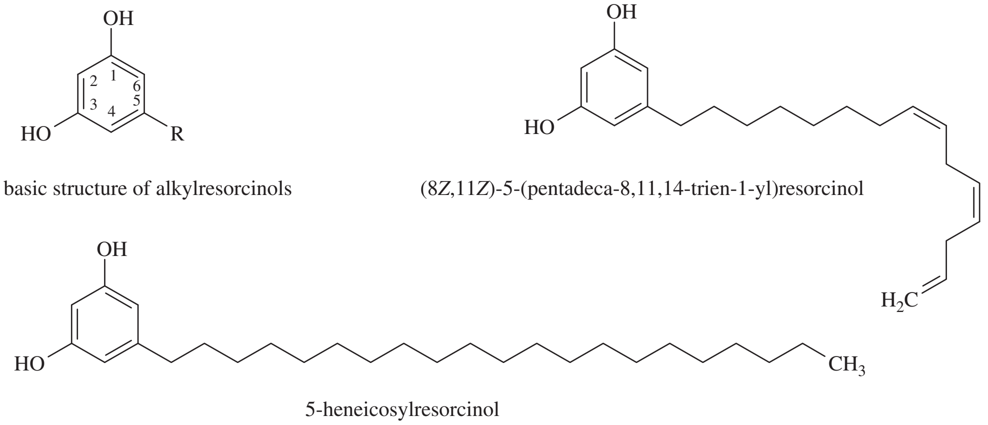
10.2.3.4 Alkylresorcinols

The term alkylresorcinols is used collectively for the group of 5-alkyl, 5-alkenyl, 5-alkadienyl and minor 5-(2-oxoalkyl) and 5-(2-oxoalkenyl) derivatives of resorcinol (benzene-1,3-diol, **10-1**). The resorcinol substituents have straight chains with an odd number of carbon atoms. The most common substituents have 13 to 29 carbon atoms.

Alkylresorcinols occur in rye, wheat and triticale and in smaller amounts in other cereals, such as barley, and in very small amounts in oats and maize. Alkylresorcinols with alkyl chains C17:0–C25:0 are abundant in whole-grain wheat and rye. They are located mainly in embryo (germ) and the outer layer of the kernel; therefore they remain predominantly in the bran during kernel milling. Their content in wheat is 200–1429 mg/kg (around 2000 mg/kg in the germ). Durum wheat (*Triticum durum*) content of alkylresorcinols is on average 455 mg/kg and the average relative homologue composition is C17:0 (0.4%), C19:0 (14%), C21:0 (58%), C23:0 (21%) and C25:0 (6.5%). The level of alkylresorcinols in rye kernels is 720–2000 mg/kg (in the germ about 3000 mg/kg) and in triticale it is between the values for rye and wheat, The main compound in wheat, rye and triticale is 5-heneicosylresorcinol (**10-1**) and in barley is 5-pentacosylresorcinol. Alkenylresorcinols represent about 30% of the content of alkylresorcinols in rye and about 10% in wheat. The content of alkylresorcinols is significantly reduced during dough fermentation and baking. In extruded cereal products, the original amount of alkylresorcinols is reduced to about 50–80%.

5-Alkyl, 5-alkenyl and 5-alkadienyl resorcinols also occur as allergenic compounds in mango (*Mangifera indica*, Anacardiaceae). Depending on cultivar, their contents range from about 80 to 1850 mg/kg of dry matter in mango peels and from about 5 to 190 mg/kg of dry matter in mango pulp. The profile of these substances was found to be highly characteristic, with an average homologue composition of C15:0 (6.1%), C15:1 (1.7%), C17:0 (1.1%), C17:1 (52.5%), C17:2 (33.4%), C17:3 (2.4%), C19:1 (2.1%) and C19:2 (0.8%).

Alkylresorcinols in cereals negatively influence the growth of animals. Their toxicity decreases with increasing length of the substituent and grows with the number of double bonds in the chain. The most toxic are homologues with unsaturated and shorter (13 or less carbon atoms) chains. The toxicity of alkylresorcinols is based on influencing the hydrophobic properties (permeability) of membranes for potassium ions and some organic compounds (such as glycerol). Alkenylresorcinols also exhibit haemolytic activity. Some synthetic substituted resorcinols (e.g. 4-hexylresorcinol approved as a food additive in the EU, E586) proved to be inhibitors of enzymatic browning reactions, causing blotches on canned shrimps.



10-1, structures of substituted resorcinols

Toxic 5-alkatrienyl, 5-alkadienyl, 5-alkenyl and 5-alkyl resorcinols, collectively known as cardol, occur in the cashew nutshell liquid (CNSL), also known as cashew shell oil, a natural resin found in the honeycomb structure of the cashew nutshell. It is a byproduct of cashew nuts processing (see Section 8.2.6.1.6). A typical solvent-extracted CNSL contains 15–20% of cardol. The main components are (8*Z*,11*Z*)-pentadeca-8,11,14-trien-1-yl (**10-1**), (8*Z*,11*Z*)-pentadeca-8,11-dien-1-yl, (8*Z*)-pentadeca-8-en-1-yl and pentadecyl derivatives.

10.2.3.5 Tannins

Antinutritional substances also include tannins, which are found in relatively large quantities in the seeds of leguminous plants (in amounts up to 0.45 g/kg in soya beans and 20 g/kg in common beans) and in oilseed extraction meals. Their complexes and reaction products with proteins are resistant to enzymatic hydrolysis, which results is lower digestibility and subsequently in reduced weight gain in livestock. Excessive consumption of tannins may also lead to decreased absorption of some minerals and can cause damage to the intestinal mucosa.

10.2.3.6 Carbohydrates

A special group of antinutrients are α-galactosides also called α-D-galactosides of saccharose. They are found in a significant number in legumes, where they have a role as storage carbohydrates. An important representative of these sugars is trisaccharide raffinose and its higher homologues, while a less common compound is trisaccharide manninotriose (Table 4.16) and α-galactosides of cyclitols that are classified as pseudooligosaccharides (see Section 4.3.1.2.2). The presence of these substances in legumes limits to some extent the use of legumes for human consumption and in nutrition of monogastric animals, because their flatulent activity results in gastrointestinal discomfort. In ruminants, these problems do not occur because α-galactosides are hydrolysed by α-galactosidase.

10.3 Toxic compounds

Natural toxic substances can be classified into two major groups:

- substances causing food intolerance that are toxic only to certain individuals
- toxins that are toxic to all individuals.

10.3.1 Food intolerance

Food intolerance is an adverse reaction of the body to some food or food ingredient that occurs every time the food is eaten, but particularly if larger quantities are consumed. It is estimated that about 0.3–7% of the population suffer from food intolerance symptoms, an abnormal physiological reaction to an accepted food, usually because the body does not produce enough of the particular chemical or enzyme that is needed for that food's digestion. The unpleasant symptoms of food intolerance include nausea, bloating, abdominal pain and diarrhea, which can begin hours or days after eating or drinking the food in question, but are not usually life threatening.

Food intolerance does not include psychological reactions to food and eating disorders, such as *anorexia nervosa* and *bulimia nervosa*. *Anorexia nervosa*, commonly referred to simply as anorexia, is an eating disorder that consists of the complete opposition of a person to eating. It makes the person sick or constantly feeling hunger-free, which can lead to malnutrition and other problems associated with starvation. *Bulimia nervosa*, also called bulimia, is an eating disorder that consists in seizure eating and attempts to vomit (purge) the food by inducing diarrhea, use of appetite suppressants or other substances to control weight loss.

Conditions resulting in food intolerance caused by non-immunological reactions of the organism include metabolic disorders, sensitivity to certain food (anaphylaxis), aversion to certain food (idiosyncrasy) and poisoning manifested by similar symptoms as allergies. The cause of these metabolic disorders of the organism is usually insufficient activity of some enzymes, which is hereditary, so food intolerance of this type is a congenital metabolic disorder. A common example is alcohol intolerance caused by deficiency of an enzyme alcohol dehydrogenase, which is common among Asian people, where about 50% are affected. Drinking even small amounts of alcohol can make affected people feel unwell. Other most common non-immunological reactions caused by insufficient activity of enzymes include:

- **lactose intolerance**
- **phenylketonuria**
- **favism**.

A well-known type of hypersensitivity to food (anaphylaxis) is a reaction to strawberries, which is manifested by urticaria (hives). Among the manifestations known as aversion to food (idiosyncrasy), whose mechanism is not yet known, is the so-called Chinese restaurant syndrome (associated with a higher intake of sodium-hydrogen glutamate) and migraine occurring after eating certain foods. An ordinary poisoning presenting as an allergy is histamine-induced poisoning, which is caused by eating some fish or fermented products with higher content of histamine (such as smoked tuna and wine).

10.3.1.1 Lactose intolerance

Lactose intolerance, often confused with milk allergy, is one of the most common metabolic disorders. Under normal conditions, lactose in the digestive tract is hydrolysed by β-galactosidase (lactase) to glucose and galactose, which are then used as an energy source. In the case of lactose intolerance, this key enzyme in the body is missing or not very active. Disaccharides are generally not absorbed by the wall of the small intestine, so in the absence of lactase, lactose

passes from food into the colon, and is fermented there by intestinal bacteria, similarly to other non-utilisable carbohydrates, forming gases (hydrogen, carbon dioxide and methane). Three types of lactose intolerance are recognised:

- primary lactose intolerance, which is common among non-breastfed children in many Asian and African countries;
- secondary lactose intolerance, in which lactase is not biosynthesised as a result of various gastrointestinal diseases, such as giardiose (intestinal parasitic disease caused by the flagellate *Giardia intestinalis*), viral diseases, acute gastroenteritis (intestinal flu), in which the person is vomiting, experiencing diarrhea and high temperature;
- congenital lactose intolerance, a genetic disorder in which lactase is not biosynthesised from birth (since it is a metabolic disorder, the disease is lifelong, but may show a slight improvement with age).

Lactose intolerance also increases with age. It is estimated that globally about 75% of the adult population shows a decrease in lactase activity during adolescence and adulthood (in Northern Europe about 5% of the population, in southern Europe about 71% and in some African and Asian countries up to 90%).

Elimination of milk, or accordingly a control of the diet containing milk, is the best way to prevent this metabolic disorder. Lactose intolerance can be avoided through the use of appropriate technological procedures. One of them is enrichment of milk by β-galactosidase and milk fermentation. Fermented milk products largely contribute to reducing adverse patient reactions to milk as much of the lactose present in milk is converted into lactic acid. Foods with low lactose intended for consumption have to contain up to 10 g/kg (or 10 g/l) lactose, lactose-free foods can contain only 100 mg/kg (or 100 mg/l) lactose and no galactose.

Lactose intolerance is secondarily manifested in individuals using antidepressant drugs by digestive problems caused by biogenic amine tyramine.

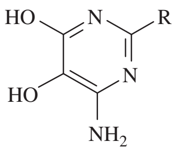
10.3.1.2 Phenylketonuria

Phenylketonuria is a congenital (a condition that is present at birth) metabolic disorder consisting in the disruption of the metabolism of the amino acid phenylalanine, which is a component of all proteins. Very low activity or absence of phenylalanine hydroxylase enzyme, which breaks down phenylalanine, is caused by mutations (damage) of the gene for this enzyme. If this condition is not treated, phenylalanine metabolites, such as phenylpyruvic, phenyllactic and phenylacetic acids (urine of patients has a characteristic mice-like odour), accumulate in the body and cause total disruption of child development. The result is damage to the development of the nervous system, leading to mental disability (microcephaly) manifested by termination of the growth of the brain and delayed psychomotoric development. The prevalence of phenylketonuria shows considerable geographic variation. It is estimated to be present in 1/10 000 live births in Europe and the United States, with a higher rate in some countries (Ireland and Italy). Prevalence is particularly high in Turkey, where it is 1/4000 live births, but it is far rarer in the Finnish, African and Japanese populations.

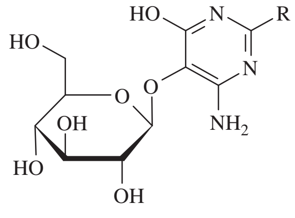
Phenylketonuria of children cannot be cured, and so, as in the case of celiac disease or lactose intolerance, they need to follow a controlled diet. The diet is based on nutrition with a reduced content of natural proteins. The phenylalanine content in foods cannot exceed 200 mg/kg (200 mg/l). The aim is to keep the level of phenylalanine in the blood at the lowest possible level. Reducing protein intake depends on the individual and is based on the degree of tolerance to phenylalanine (below 30% compared wiht the general population). According to the age and nutritional needs of the person, the natural protein can be replaced by an artificial mixture of individual amino acids without phenylalanine, but the use of a mixture of phenylalanine-free higher peptides is more advantageous with respect to sensory and osmotic properties. Higher peptides can be prepared by plastein synthesis (partial protein hydrolysis, treatment with protease under suitable conditions leading to release of hydrophobic amino acids, plastein reaction in the presence of added tryptophan and tyrosine, which yields practically phenylalanine-free plastein).

10.3.1.3 Favism

Favism is caused by the toxic pyrimidines divicine (2,4-diamino-5,6-dihydroxypyrimidine) and isouramil (4-amino-2,5,6-trihydroxypyrimidine, **10-2**), which occur in broad (fava) beans (*Vicia faba*, Fabaceae) and horse beans (*V.f.* var. *equina*), and in small quantities in some other legumes as 5-*O*-β-D-glucopyranosides known as vicine and convicine (**10-3**), respectively. These glycosides probably offer some protection against elicitors (bacteria and fungi) as they exhibit bacteriostatic and fungistatic effects. Sweet pea (vetchlings) (*Lathyrus* spp.) and pea (*Pisum* spp.) species do not contain pyrimidine glycosides. The vicine content of fava beans generally ranges from 4.2 to 10.8 g/kg, while the convicine content tends to be lower, within 0.3 to 0.5 g/kg. Divicine and isouramil arise from the respective glucosides, mainly in the large intestine, by hydrolysis with microbial β-glucosidases.



10-2, divicine, R = NH_2
isouramil, R = OH



10-3, vicine, R = NH_2
convicine, R = OH

The main favism symptom is acute haemolytic anaemia, accompanied by high fever, jaundice and swelling of the liver and spleen, as toxic pyrimidines oxidise the reduced form of glutathione in erythrocytes. Favism is manifested especially in individuals with low (usually hereditary) activity of the enzyme glucose 6-phosphate dehydrogenase in erythrocytes that reduces the oxidised form

of glutathione. This results in a lower concentration of reduced glutathione, which has a protective effect on the erythrocyte membrane. In addition to active substances of fava beans, medical drugs that oxidise glutathione can also invoke favism. The addition of vitamin A, C and E and chelating agents partially protects the body against favism. The glycoside content in the beans can be reduced by extraction with water. In this way it is possible to remove 80–90% of the original amount of glycosides in beans.

The presence of fava beans in feed mixtures for laying hens negatively affects the quality of eggs, because the eggs have a lower weight and increased yolk fragility.

10.3.2 Food allergy

Food intolerance, often called a pseudo-allergic reaction, is not the same as a food allergy, because it does not involve the immune system. Food allergy is an abnormal response of the immune system to even a tiny amount of a particular food, which is manifested by symptoms such as sneezing, itching, nasal obstruction (hay fever), redness of the eyes (conjunctivitis), nausea, diarrhea, vomiting, eczema, coughing and wheezing (asthma). Because the body is reacting to something that is otherwise harmless, this type of allergic reaction is often called a hypersensitivity reaction. Rarely, allergic reactions to food can sometimes cause serious illness and death, such as a life-threatening set of symptoms called anaphylaxis, or anaphylactic shock. It is estimated that about 2.5% of adults and about 6–8% of children, mainly younger than 6 years, have true food allergies.

Recognised are allergies induced by immunological reactions of the organism caused by **allergens** that cause production of immunoglobulin E (IgE), and allergies that do not cause the production of IgE.

10.3.2.1 Allergens causing abnormal production of IgE

Allergens, the agents causing the allergy (in immunological term antigens), may be proteins, polysaccharides and low molecular weight compounds called **haptens**. A food allergy is an allergic reaction of the organism when the allergen is a component of food. The most common manifestations of an allergy are skin reactions (such as atopic eczema, hives and swelling), respiratory system reactions (rhinitis) or reactions of digestive system (vomiting, diarrhea and abdominal pain). Allergens acquire immunological properties by interactions with serum proteins of the organism. Immunogenicity of proteins that are hosts for foreign substances is given by the amino acid sequences and their constitution and conformation. The mechanism of allergies takes place in two stages. The first stage is a specific immunological reaction, in which the allergen reacts with antibodies (called immunoglobulin E, in short IgE) with the release of mediators (such as prostaglandins and biogenic amines, one of which is histamine). In the second, non-specific stage, the hypersensitive organism responds to mediator stimulation by pathological manifestations. Hapten is a non-proteinaceous substance that acts as an antigen by combining with particular bonding sites on an antibody. Unlike a true antigen, it does not induce the formation of antibodies.

In theory, any food can be an allergen, but all foods do not have the same ability of allergising the organism. Among the foods that tend to cause the most allergic problems are milk, eggs (egg white), some fish, some fruits (apples, pears, apricots, peaches and strawberries), some vegetables (tomatoes, celery, spinach and parsley), cereals (hypersensitivity to cereals usually persists to a certain stage of life and improves with age), legumes (such as soya beans), seeds and nuts (sesame seeds and peanuts; reactions are usually very severe).

Allergens are substances that are very thermostable and stable in acidic media, so they resist the digestive processes. The most common ways to reduce allergenicity is the enzyme hydrolysis of proteins and their thermal denaturation. One of the modern ways to reduce allergenicity is inhibition of hyaluronidase, one of the target enzymes that control allergic responses, by pectin. Its inhibition is thus an indicator of antiallergic activity. Tea extracts have similar effects as pectin, inhibiting hyaluronidase.

The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee was founded in 1984 to establish a system for nomenclature of allergens and their characterisation.

10.3.2.1.1 Milk

Allergic symptoms have been reported for casein fractions and serum (whey) globulins of cow's milk. The most important allergens include α- and β-casein and β-lactoglobulin. Allergic effects have also been seen in α-lactoglobulin and α-lactalbumin.

Allergenicity of milk proteins can be reduced to some extent by enzymatic hydrolysis of proteins, their thermal denaturation or combined effects of both procedures. For the same reasons, fermented dairy products show a reduced allergenicity. Enzyme cleavage of casein can yield fragments with a relative molecular weight lower than 1000 Da with reducing allergenicity, but this procedure may also produce bitter peptides. The most commonly used proteases are proteases of the human gastrointestinal tract (pepsin, trypsin and chymotrypsin). Better performance is achieved by enzymatic hydrolysis in combination with thermal denaturation, but caseins are very resistant to thermal denaturation. A reduction of milk allergenicity can be also achieved by ultrafiltration, in which the high molecular weight allergens resistant to denaturation and hydrolysis are removed. By combining enzymatic hydrolysis, thermal denaturation and ultrafiltration, the allergenicity can be reduced by about 1000 times.

10.3.2.1.2 Eggs

Egg allergy accounts for one of the most prevalent food hypersensitivities in industrialised countries. The estimated prevalence of egg allergy varies between 1.6 and 3.2% and, thus, makes it the second most common cause of food allergy in children. The majority of the relevant egg allergens (Gal d 1 to Gal d 6) have been identified in the egg white: Gal d 1 (ovomucoid, 28 kDa), Gal d 2 (ovalbumin, 44 kDa), Gal d 3 (ovotransferrin, 78 kDa) and Gal d 4

(lysozyme C, 14 kDa). Allergens Gal d 5 (serum albumin, 14 kDa) and Gal d-6 (YGP42, 35 kDa) are allergens found in the egg yolk.

10.3.2.1.3 Cereals

Cereal proteins are not significant as allergens. For example, buckwheat (*Fagopyrum esculentum*, Polygonaceae) contains thermostable allergens Fag e 2 (2S albumin, 16 kDa) and Fag e 3 (vicilin fragment, 19 kDa), but some allergenicity was even observed for rice (containing allergens Ori s 1 and Ori s 12), barley (Hor v 1 identical to Hor v 15, Hor v 12, Hor v 16, Hor v 17 and Hor v 20) and wheat (Tri a 12, Tri a 14, Tri a 15, Tri a 18, Tri a 19, Tri a 21 and Tri a 25 to Tri a 37). The disease caused by wheat allergens is called Duhring's disease or *dermatitis herpetiformis* and is different from celiac disease. Wheat flour may cause baker's asthma, one of the most common forms of occupational asthma. This allergic disease is caused by inhalation of flour dust containing proteins of relative molecular weight of around 15 kDa, which act as α-amylase inhibitors in the grain. It can affect workers in bakeries, flour mills and kitchens.

10.3.2.1.4 Legumes

Soya is one of the most important sources of food allergens (Gly m 1-Gly m 7) that are responsible for about 90% of allergic reactions in the child population. The allergen Gly m 1 is a hydrophobic protein, Gly m 2 is a small cysteine-rich cationic protein (defensin), Gly m 3 is an actin-binding protein (profiling), Gly m 4 is a member of the PR-10 proteins family, Gly m 5 is β-conglycinin (vicilin, 7S globulin), Gly m 6 is is glycinin (legin, 11S globulin) and Gly m 7 is a seed biotinylated protein. Reduction of allergenicity can be achieved by thermal denaturation or enzymatic hydrolysis of proteins. Some soy proteins are relatively heat-stable, so that the complete elimination of allergenicity just by heating is impossible. Enzymatic hydrolysis with subsequent thermal denaturation is an appropriate way of producing hypoallergenic soya protein hydrolysates. Soy proteins are often cross-linked by disulfide bonds. To reduce allergenicity, these bonds must be cleaved, for example with *N*-acetylcysteine. Carbohydrates reduce the allergenicity of soy proteins by modifying the molecules in locations that are responsible for allergenicity. The reaction products of soya proteins with oxidised lipids increase the allergenicity, but oxidised soybean oil does not cause any allergic response.

10.3.2.1.5 Nuts

Peanuts contain several allergens (Ara h 1-Ara h 13) that are highly resistant to heat. The three major ones (Ara h 1-Ara h 3) are members of the cupin superfamily of proteins (Ara h 1 and Ara h 3, containing a conserved barrel domain, cupa is the Latin term for a small barrel), to the 7S and 11S globulin families, respectively, and Ara h 2 is a conglutinin related to 2S albumins. Peanut allergy (affecting 1.3% of the general population) is the most common cause of severe allergy attacks, especially in children, and appears to be on the rise. Peanut allergy symptoms can range from a minor irritation to a life-threatening reaction (anaphylaxis). For some people with peanut allergy, even tiny amounts of peanuts can cause a serious reaction.

10.3.2.1.6 Other foods

Allergenicity has been demonstrated in a number of other plant materials, exemplified by green peas, rapeseed, mustard, sesame seed and other materials. The common characteristics of these allergens are low molecular weight and high content of cysteine/cystine. Methods for reducing their allergenicity have not been found. Owing to the high content of disulfide bonds in the molecule, these allergens are resistant to hydrolysis by proteases.

10.3.2.2 Other allergens

10.3.2.2.1 Coeliac disease

Coeliac disease (also known by the Latin name *coeliac sprue*), non-tropical sprue or gluten enteropathy is an autoimmune malabsorption disorder of the small intestine caused by sensitivity to gluten (a protein found in wheat) and to similar proteins in rye, barley and oats. The disease is caused by gliadine (prolamine) and gluteline fractions of wheat protein (called gluten) or gluteline fractions of barley (hordeine) and rye (secaline) having two amino acid sequences: Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro. Coeliac disease does not cause abnormal production of IgE.

Coeliac disease may occur in genetically predisposed people of all ages from middle infancy onward. The prevalence of coeliac disease in different countries varies considerably. The global average is about 1/3350. In children, celiac disease usually occurs soon after they are first given a diet containing gluten (semolina porridge, biscuits or soup thickened with flour). Infants, toddlers and young children may often exhibit growth failure, vomiting, bloated abdomen, behavioural changes and failure to thrive. In adults, celiac disease can be triggered for the first time between 30 and 50 years of life after surgery, viral infection, severe emotional stress, pregnancy or childbirth. It can manifest similarly as in children, but often there are cases with less developed symptoms.

At present, the only sure way to cure celiac disease is to follow a gluten-free diet for life, avoiding wheat, rye and barley. As alternatives, products containing millet, maize, rice, amaranth, buckwheat, soybeans and edible chestnuts may be consumed. Vegetables, including potatoes, fruits, meat, fish, eggs, milk and milk products are also allowed.

The EU adopts common rules concerning the composition and labelling of foods for people who are intolerant to gluten. Very low gluten foods containing ingredients made from wheat, rye, barley, oats or their crossbred varieties that have been especially processed to reduce gluten, must not contain a level of gluten exceeding 100 mg/kg. In order to be labelled 'Gluten-free', products must not exceed 20 mg gluten content per kilogram.

10.3.3 Toxins

Toxins are produced by microorganisms, plants and some animals. They are classified according to various criteria, such as structure,

biological effects, origin and so on, but so far there is no clear system of classification. Classification according to the structure of toxins is thus largely applied in this chapter, but in some cases this system is also combined with classification according to origin (occurrence) and biological effects. Not all toxic compounds are included. Many others are described in sections devoted to amino acids, peptides, proteins, lipids, carbohydrates, minerals, flavourings, pigments, food additives and contaminants. No attempt has been made to cover toxic compounds that are primarily of pharmaceutical and medicinal interest, nor has an attempt been made to cover toxic compounds accidentally ingested or taken as illicit drugs.

10.3.3.1 Alkaloids

Alkaloids are a heterogeneous group of substances comprising more than 10 000 compounds of different structures. Alkaloids are basic nitrogenous compounds produced as secondary metabolites that exhibit a variety of biological effects, depending on the amount consumed. They often occur as a mixture of compounds of related structures, either as free compounds, *N*-oxides, salts of carboxylic acids, their esters or amides, or as glycosides. Some products are considered as detoxification plant products, growth regulators, and spare forms of nitrogen that may have an important role in protecting plants against elicitors and pathogens and in the evolution of plant species.

Alkaloids are found in about 15–20% of vascular plants, in seeds, leaves, roots, bark and other parts, but they similarly occur in certain species of mosses, fungi, bacteria and some invertebrates (centipedes, beetles, butterflies and crustaceans) and vertebrates (e.g. in frogs and salamanders). Alkaloids of certain species of insects and other animals are often of plant origin, but may also be synthesised *de novo*.

No evaluation criterion exists that would allow the characterisation of alkaloids as a single group of natural substances. Some alkaloids are also classified as herbal antibiotics (natural pesticides), because they are a part of the defence mechanisms of plants against elicitors and predators. Some alkaloids are classified as natural toxic amino acids (such as ibotenic acid and agaritine), biogenic amines (e.g. histamine, hordenine or psilocin) and natural dyes (betalaine or berberine). Many alkaloids and related compounds also arise during thermal processing of foods from essential nutrients. For example, a number of indole alkaloids are produced in the Maillard reaction, such as β-carbolines (see Section 2.5.2.3.1), quinoline and quinoxaline derivatives and other biologically active nitrogen heterocycles, such as aminoimidazoazaarenes.

Alkaloids are normally classified into three main basic groups:

- **true alkaloids**
- **pseudo alkaloids**
- **protoalkaloids**.

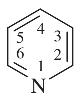
True alkaloids are typically heterocyclic nitrogen bases derived from amino acids. They exhibit a wide range of physiological effects and are often highly toxic to humans and other animals (such as nicotine in tobacco). Pseudo alkaloids are heterocyclic nitrogen bases, nevertheless their precursors are not amino acids, but terpenoids or purines. They are generally less toxic than true alkaloids. Examples of pseudo alkaloids are caffeine in coffee and solanine in potatoes. Protoalkaloids are basic amines derived from amino acids, but the nitrogen is not a part of the aromatic (heterocyclic) system. An example is capsaicin present in hot peppers.

Common to all three groups is the classification of the individual alkaloid groups according to the heterocyclic compounds from which the basic skeletons are derived. True alkaloids with a pyridine ring are, for example, nicotine and many other tobacco alkaloids. Besides the pyridine ring, these alkaloids also contain pyrrolidine and piperidine rings. The black pepper alkaloid piperine is also derived from the piperidine ring. Many alkaloids of medicinal plants called necines are derived from pyrrolizidine, lupine alkaloids are derived from chinolizidine, quinine from the bark of the cinchona tree is derived from quinoline and berberine found in barberry and other plants is derived from isoquinoline. Pseudo alkaloids, such as caffeine in coffee and tea, are derived from purine, glycoalkaloids containing nitrogen in the molecule, such as solanine in potatoes, are derived from steroids. Protoalkaloids of hot peppers are derivatives of vanillic acid amide, called capsaicinoids.

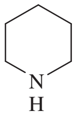
Another aspect is also frequently applied, which is the alkaloid origin. Nicotine, for example, is included among the tobacco alkaloids and quinolizidine alkaloids occurring in different types of lupine are lupine alkaloids. For practical reasons, classification based on these principles has also been used in the following sections. Today, classification according to the precursors of alkaloid biosynthesis is often promoted. This, however, requires a detailed knowledge of the biogenesis mechanisms (Table 10.2).

10.3.3.1.1 Pyridine alkaloids

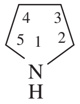
The most important alkaloids, which contain the pyridine ring (**10-4**) in the molecule, are tobacco alkaloids. Besides the pyridine ring, tobacco alkaloids also contain some 1,2,5,6-tetrahydropyridine (piperidine, **10-5**) and pyrrolidine (**10-6**) rings.



10-4, pyridine



10-5, piperidine



10-6, pyrrolidine

Structure, nomenclature and occurrence

The main alkaloid of different cultivars of commercial tobacco species (*Nicotiana tabacum* and *N. rustica*, Solanaceae) is nicotine, (*S*)-1-methyl-2-(pyrid-3-yl)pyrrolidone or (*S*)-3-(1-methylpyrrolidin-2-yl)pyridine, **10-7**. Nicotine is also present in small quantities in other plants (about 24 species of 12 plant families), but especially in plants of the nightshade family, which also includes potatoes, tomatoes and eggplants (aubergines). Biosynthesis of nicotine takes place in the roots of plants, from where nicotine is transported to the aerial parts, especially to the leaves.

Table 10.2 Overview of important alkaloids occurring in foods.

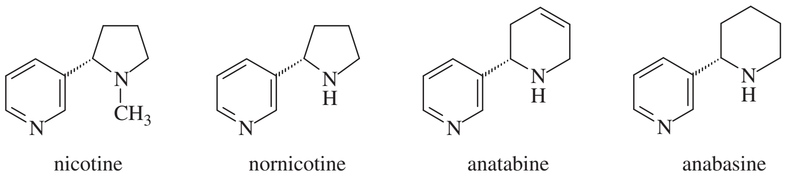
Structural types of basic skeleton	Precursors	Occurrence	Examples
True alkaloids			
Pyridine, piperidine and pyrrolidone alkaloids[a]	Arg, Lys, Orn, nicotinic acid	Tobacco leaves	Nicotine, nornicotine, anatabine, anabasine
	Lys, Phe	Black pepper seeds	Piperine
Pyrrolizidine alkaloids[b]	Arg, Ile, Leu, Orn, Val, Thr	Ragworts, groundsels	Senecionine
Nortropane alkaloids	Putrescine	Potatoes, tomatoes	Calystegines
Quinolizidine alkaloids	Lys	Lupin seeds	Lupanine, lupinine, sparteine
Quinoline alkaloids[c]	Trp, mevalonic acid	Cinchona bark	Quinine, quinidine
Protoalkaloids			
Capsaicinoids (vanillylamides)[d]	Leu, Phe, Val, malonyl-CoA	Chili pepper	Capsaicine, nordihydrocapsaicine, homodihydrocapsaicine
Pseudoalkaloids			
Purine alkaloids	Purines	Coffee, tea, cocoa	Coffeine, theobromine, theophylline
Steroid (terpenoid) glycoalkaloids	Mevalonic acid	Potatoes, tomatoes	Solanine, tomatine

[a]Pyridine ring is derived from nicotinic acid, which arises from aspartic acid and glyceraldehyde 3-phosphate via quinolinic acid; pyrrolidine ring in nicotine and nornicotinu arises from Orn, piperidine ring in anatabine and anabasine arises from Lys. Dihydropyridine ring of natural pigments betalaines arises from Tyr via DOPA and betalamic acid.

[b]Pyrrolizidine skeleton is derived from Orn. Necic acids, by which some alkaloids are esterified, such as senecic acid, arise from Ile.

[c]Precursors are Trp and monoterpenic glycoside loganin. Not listed are numerous other alkaloids derived from Trp (up to about a quarter of all alkaloids), such as indole protoalkaloids tryptamines that are covered under biogenic amines (e.g. gramine) as well as hordenine arising from Tyr. Excluded are also toxic components of higher fungi (such as psilocin) and toxic byproducts of amino acids (such as β-carbolins). Phe is a precursor of vanillylamine.

[d]The fatty acid in capsaicine is derived from Val.



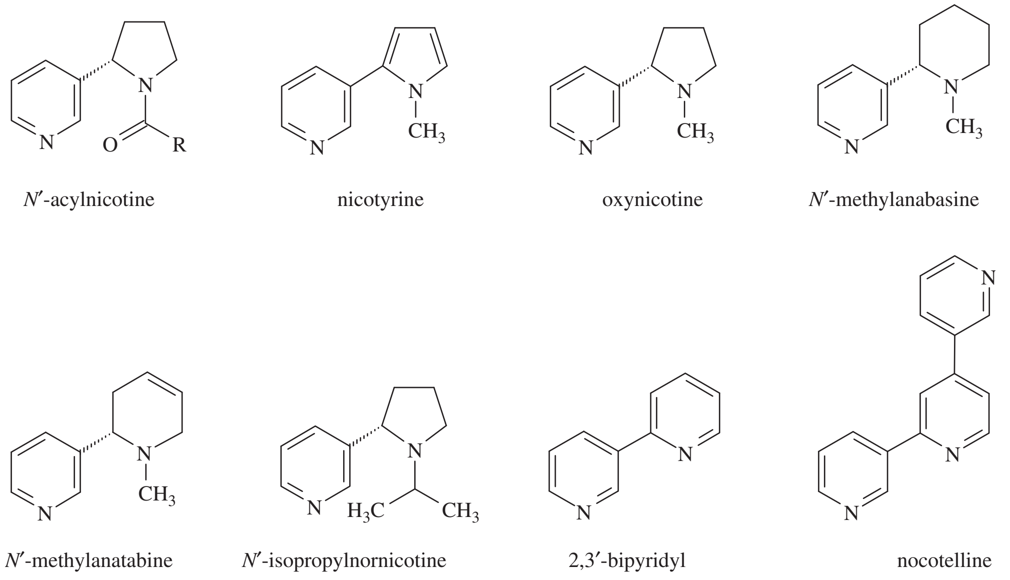
10-7, main tobacco alkaloids

Nicotine in tobacco is always accompanied by three other prominent alkaloids: nornicotine, (*S*)-2-(3-pyridyl)pyrrolidone, anatabine, (*S*)-2-(pyrid-3-yl)-1,2,5,6-tertrahydropyridine and anabasine, (*S*)-2-(pyrid-3-yl)piperidine (**10-7**). Apart from these main alkaloids, more than 20 other minor tobacco alkaloids (**10-8**) have been identified.

The total content of alkaloids in tobacco ranges from 0.3 to 3% of dry matter, depending on the species and varieties of plants, climatic and soil conditions and other factors. Approximately 95% of this amount is represented by nicotine. For example, leaves of the so-called bright-leaf tobacco, commonly known as Virginia tobacco (*N. tabacum*), with a total alkaloid content of 1.93%, containing 1.85% of nicotine, contains 0.04% of anatabine, 0.03% of nornicotine and 0.01% of anabasine. The content of other (minority) alkaloids is less than 0.01%.

Reactions and changes

The content of nicotine and other alkaloids in fresh tobacco leaves decreases slightly during post-harvest treatment, drying and fermentation. During tobacco drying and fermentation, nicotine produces nornicotine through transmethylation, which partially decomposes to nicotinic acid via myosmine. Anabasine and anatabine are formed as products of nicotine catabolism (Figure 10.1). In tobacco products, the content of alkaloids does not change. One cigarette (about 0.8 g of tobacco) contains 1 mg



10-8, minor tobacco alkaloids

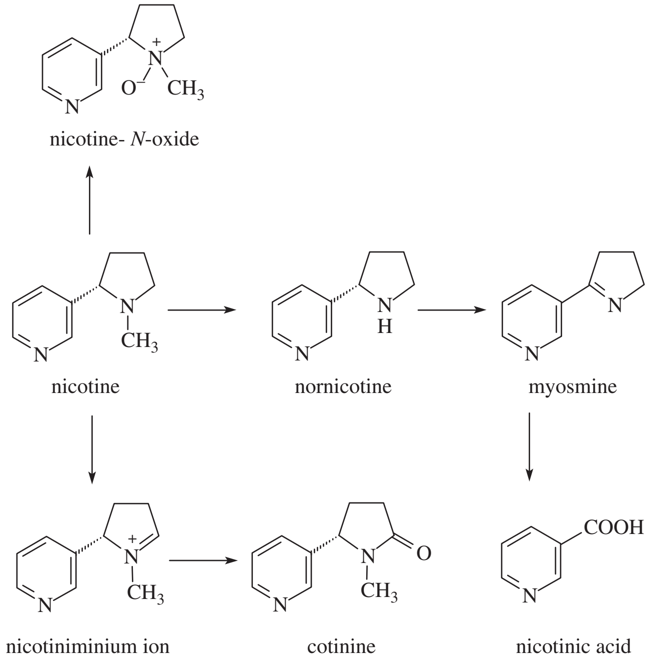


Figure 10.1 Products arising from nicotine during tobacco fermentation.

of absorbable nicotine and new products with reduced nicotine content (light, superlight and ultra light cigarettes) usually have a nicotine content of from 0.1 to 0.8 mg.

During tobacco burning, nicotine is partially oxidised to cotinine and oxynicotine that rapidly decomposes at higher temperatures. Pyrolysis of nicotine also produces hydrogen cyanide (0.004–0.270 mg per one cigarette) and other products. The resulting nitrogen oxides react with the decomposition products of nicotine to form carcinogenic nitrosamines.

Biological effects

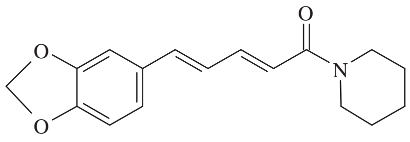
Nicotine is a potent neurotoxin, particularly for insects. In the past, a tobacco extract was used as an insecticide. In mammals, nicotine in low concentrations acts as a stimulant, but it is also the main addictive component of tobacco, which is absorbed by the body primarily from smoke. Smaller volume tobacco products are snuff and chewing tobacco. Moderate nicotine doses increase respiratory and motor activity and lead to vomiting, high doses results in tremors and end in coma. The sensitivity of animals to nicotine ranges from 6 to 30 mg/kg. Somewhat lower toxicities are exhibited by nornicotine and anabasine. Anabasine (and probably also anatabine) show teratogenic effects. Chronic pathological changes in the respiratory system and carcinogenicity of tobacco smoke have been clearly demonstrated; therefore Directive 90/239/EEC established maximum limits for the carcinogenic tar, nicotine and carbon monoxide yields of cigarettes marketed in the EU Member States. Since 1 January 2004, the amount per cigarette must not be greater than 10 mg for tar, 1 mg for nicotine and 10 mg for carbon monoxide.

10.3.3.1.2 Piperidine alkaloids

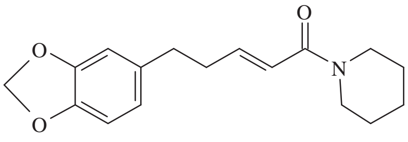
The most important alkaloids derived from piperidine (**10-6**) are alkaloids of black pepper (*Piper nigrum*, Piperaceae).

Structure, nomenclature and occurrence

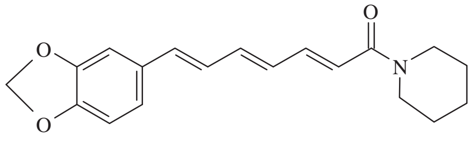
The main hot component of black pepper is piperine, piperic acid amide or (2*E*,4*E*)-piperoyl-1-piperidine or (2*E*,4*E*)-5-(1,3-benzodioxol-5-yl)-*N*-piperidinylpenta-2,4-dienamide (**10-9**). Black pepper also contains a number of related compounds as minor components, such as piperanine (**10-9**) and *N*-(3,4-methylendioxyphenyl)acylpiperidines with C_3, C_5, C_7 and C_9 acyls and the corresponding pyrrolidines, such as piperettine, piperyline, piperoleine A, piperoleine B (**10-9**) and other compounds.



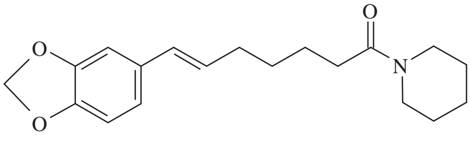
piperine



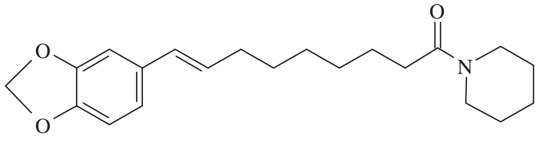
piperanine



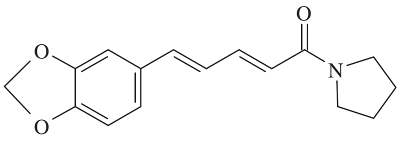
piperettine



piperoleine A



piperoleine B



piperyline

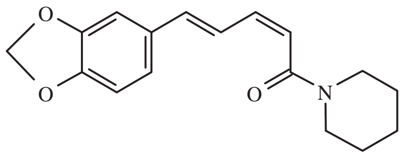
10-9, piperine and related alkaloids of black pepper

Many other unsaturated amides have been identified in *Piper* species from all tropical regions of the world.

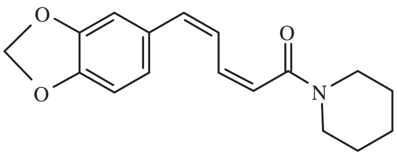
Piperine content is typically 3–8% (and sometimes more), but it depends on the origin and other variables. The highest amount of piperine in pepper fruits is just before full maturity. Green pepper, obtained from unripe fruits, therefore contains a considerable amount of piperine, as well as black pepper, which is obtained by fermentation of green seeds (see Section 8.3.6.2). The content of piperine in white pepper, which is obtained from ripe red seeds with the coloured skin removed, is somewhat lower. The level of minor alkaloids piperyline and piperettine is only 0.2–0.3% and 0.2–1.6%, respectively. The piperine content in oleoresins is normally 35–40%, which corresponds to minced spice in a ratio of about 1 : 25.

Reactions and changes

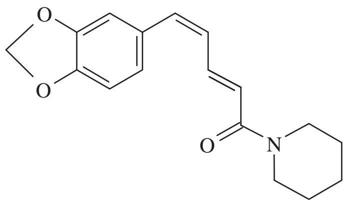
Piperine is very stable in intact seeds and in ground seeds. If protected from light, piperine is stable for at least 6 months if stored in paper and for 10 months in aluminum packaging, but is easily oxidised in air. Piperine in solutions, in ground spice and in oleoresins is accompanied by its diastereoisomers, (2*Z*,4*E*)-piperoyl-1-piperidine (isopiperine), (2*Z*,4*Z*)-piperoyl-1-piperidine (chavicine) and (2*E*,4*Z*)-piperoyl-1-piperidine (isochavicine, **10-10**), which arise by enzyme-catalysed and/or light-induced *cis/trans* isomerisations of the double bonds of the parent piperine molecule. However, they show little or no (isochavicine) pungency. Alkaline hydrolysis of piperine yields the salt of piperic acid, (2*E*,4*E*)-5-(3,4-methylenedioxyphenyl)-2,4-pentadienoic acid, and piperidine. In



isopiperine



chavicine



isochavicine

10-10, piperine isomers

slightly acidic solutions, piperine may react with nitrites to form nitrosamines.

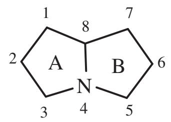
Biological effects

Piperine acts as a stimulant of the central nervous system, has weak antipyretic and mutagenic effects and inhibits some enzymes acting in the metabolism and transport of xenobiotics and drugs. Reportedly, piperine is beneficial for increasing thermogenesis (the process of generating energy in cells), which may also be helpful in reducing inflammation, improving digestion, and relieving pain and asthma. At higher concentrations, piperine damages tongue tissue, and lowers blood pressure and breathing rate. Other piperine derivatives of pepper show irritating, antimicrobial and insecticidal effects. Some related compounds in pepper exhibit antioxidant effects.

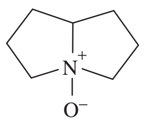
10.3.3.1.3 Pyrrolizidine alkaloids

Pyrrolizidine alkaloids are a large and important group of about 250 alkaloids produced by approximately 6000 plant species belonging to 13 families. The most important plants belong to the families Boraginaceae, Asteraceae, Heliotropiaceae, Fabaceae and Rhamnaceae. Pyrrolizidine alkaloids occur in relatively small amounts (0.1–1% of dry matter) as a complex mixture of more than ten different compounds. Important sources of alkaloids are some herbs and certain fodder plants.

The skeletons of pyrrolizidine alkaloids are derived from 2,3,5,6,7,8-hexahydro-1*H*-pyrrolizine, known as pyrrolizidine (**10-11**), and from pyrrolizidine-*N*-oxide (**10-12**). The bases of the pyrrolizidine alkaloids are necines (necine bases) derived from bicyclic amino alcohols, which have their origin in 1-hydroxymethylpyrrolizidine. Necines may be saturated or may have a double bond at C-1 of ring B and may also have an additional one or two hydroxyl groups at C-2, C-6 or C-7. Necines are esterified with carboxylic acids, which are called necic acids. Biosynthesis takes place in the roots, where the alkaloids occur as the corresponding *N*-oxides. They are then transported to the aerial parts of the plant and stored in vacuoles.



10-11, pyrrolizidine



10-12, pyrrolizidine-*N*-oxide

Structure, occurrence and terminology

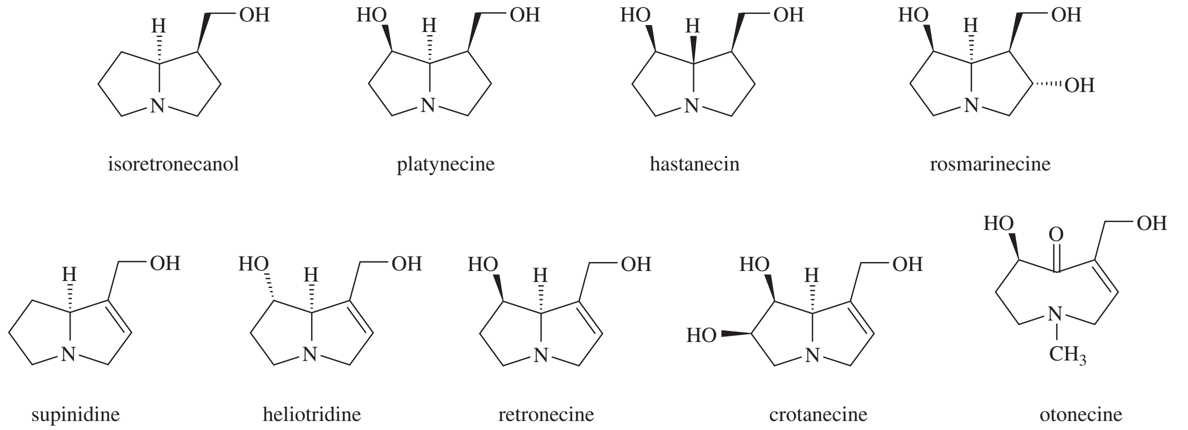
The most frequently occurring necines in medicinal plants are supinidine, isomeric retronecine and heliotridine, platynecine, hastanecine and otonecine (**10-13**).

Necine bases are esterified by different carboxylic acids. With the exception of acetic acid, these acids have 5–10 carbon atoms and include mono- and dicarboxylic acids with branched chains substituted with hydroxyl, methoxyl (other alkoxyl), epoxy, carboxy and acetoxy groups. Necines with two hydroxyls, such as 7,9-necinediol, may be esterified with carboxylic acids in positions C-7 and C-9 or in both positions. Esterification with dicarboxylic acids produces macrocyclic alkaloids with 11 to 14-membered rings. Depending on the ester type, the following alkaloids can be recognised:

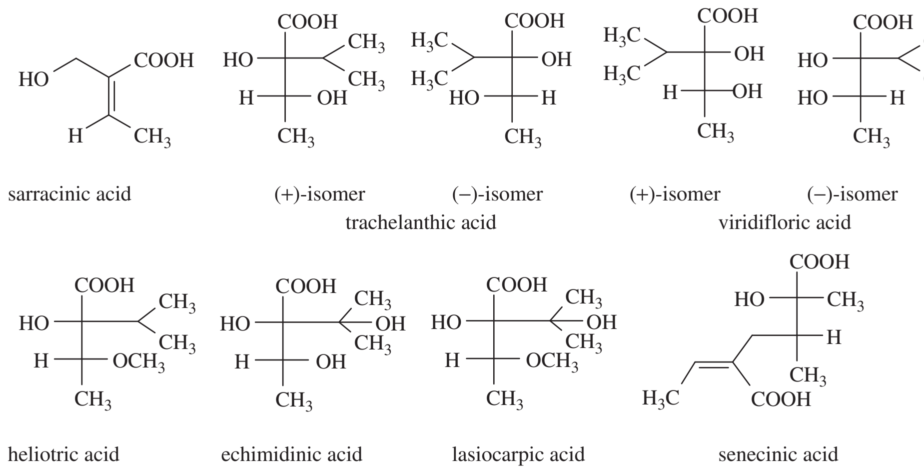
- monoesters
- diesters
- macrocyclic diesters.

The most frequently occurring carboxylic acids with five carbon atoms per molecule are (*Z*)-2-methylbut-2-enoic acid, also known as (*Z*)-2,3-dimethylacrylic or angelic acid, its isomer (*E*)-2-methylbut-2-enoic acid, known as (*E*)-2,3-dimethylacrylic (tiglic) acid, (*Z*)-3-methylbut-2-enoic acid (senecioic) acid (8-62) and (*Z*)-2-hydroxymethylbut-2-enoic (sarracinic) acid (**10-14**).

Frequent carboxylic acids with seven carbon atoms per molecule are (+)-trachelanthic acid, namely (2*S*,3*R*)-2,3-dihydroxy-2-isopropylbutanoic acid, (−)-trachelanthic acid, which is (2*R*,3*S*)-2,3-dihydroxy-2-isopropylbutanoic acid, (+)-viridifloric



10-13, necine bases



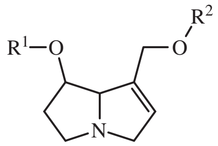
10-14, necic acids

acid, also known as (2*R*,3*R*)-2,3-dihydroxy-2-isopropylbutanoic acid, and (−)-viridifloric acid, namely (2*S*,3*S*)-2,3-dihydroxy-2-isopropylbutanoic acid (10-14). Methyl derivatives of these acids have eight carbon atoms in the molecule. For example, a methyl derivative of (+)-trachelanthic acid is (2*S*,3*R*)-2-hydroxy-3-isopropyl-3-methoxybutanoic (heliotric) acid, three hydroxyl groups in the molecule has (2*R*,3*R*)-2,3-dihydroxy-2-(1-hydroxyethyl)-3-methylbutanoic (echimidinic) acid and its methyl derivative is (2*R*,3*R*)-2,3-dihydroxy-2-(1-methoxyethyl)-3-methylbutanoic (lasiocarpic) acid (**10-14**). An example of necic acid bound in the macrocyclic diesters (having ten carbon atoms) is (2*S*,3*R*,5*Z*)-5-ethylidene-2-hydroxy-2,3-dimethylhexane-1,5-dioic acid (senecinic) acid (**10-14**).

Pyrrolizidine alkaloids are found in some medicinal herbs that are used as a traditional medicine in many countries. These plants grow mainly in temperate climates, but some require tropical or subtropical climates. The Boraginaceae family is represented by common comfrey (*Symphytum officinale*), borage (*Borago officinalis*), common bugloss (*Anchusa officinalis*), viper's bugloss or blueweed (*Echium vulgare*) and houndstongue (*Cinoglossum officinale*). The Asteraceae family includes coltsfoot (*Tussilago farfara*), butterbur (*Petasites hybridus*) and the Rhamnaceae family includes buckthorn (*Rhamnus cathartica*). For example, butterbur, used as a remedy for headache and inflammation, has the total alkaloid content in the root of about 0.01%, but the alkaloid content in other parts of the plant is negligible. The main alkaloid is senecionine.

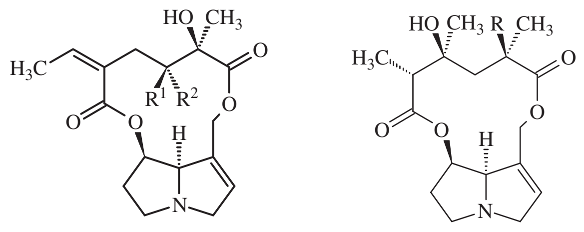
Pyrrolizidine alkaloids also contain a number of plants that can become part of the feed, and therefore may cause poisoning of livestock. Examples are the legume forage plants of the genus *Crotalaria* (Fabaceae), commonly known as rattle pods, and especially *C. spectabilis* used for green manure and as cover crops in subtropical and tropical regions of Africa, Asia and the United States. Also poisonous to cattle are some plants of the Asteraceae family, such as ragwort (*Senecio jacobaea*), hemp-agrimony (*Eupatorium cannabinum*), summer ragwort (*Ligularia debtata*) and bill goat weed (*Ageratum conyzoides*) and European heliotrope (*Heliotropium europeum*) of the Boraginaceae family.

The main comfrey alkaloids (echinatine, echimidine, heliosupine and lasiocarpine) and European heliotrope alkaloids (heliotrine, lasiocarpine and symphytine, **10-15**) formulae are shown as examples of monoesters and diesters of various necines. Echimidine is derived from retronecine; other alkaloids are derived from heliotridine. Examples of macrocyclic diesters derived from retronecine are alkaloids of ragwort senecionine and seneciphylline and alkaloids of the *Crotalaria* legumes fulvine and monocrotaline (**10-16**).



10-15, monoesters and diesters

echinatine, R^1 = H, R^2 = viridifloroyl
heliotrine, R^1 = H, R^2 = heliotroyl
echimidine, R^1 = angeloyl, R^2 = echimidinoyl
heliosupine, R^1 = angeloyl, R^2 = echimidinoyl
lasiocarpine, R^1 = angeloyl, R^2 = lasiocarpoyl
symphytine, R^1 = tigloyl, R^2 = viridifloroyl



10-16, macrocyclic diesters

senecionine, R^1 = H, R^2 = CH_3
seneciphylline, R^1 and R^2 = CH_2

fulvine, R = H
monocrotaline, R = OH

Reactions and changes

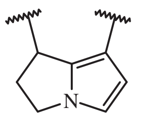
The concentration of alkaloids in toxic plants is highly variable, even between different parts of the same plant, and depends on many other factors. When preparing medicinal herbal teas, most

alkaloids leach into water. Stability of individual compounds is not known, but it can be supposed that partial hydrolysis of esters to necine bases and carboxylic acids may occur.

Biological effects

Most plants containing pyrrolizidine alkaloids are toxic to humans and domestic animals. Poisoning occurs most frequently after eating different parts of plants as food (such as salads from young leaves of comfrey and butterbur), extracts from medicinal herbs or contaminated cereals. Cattle, horses, farmed deer and pigs are most susceptible; sheep and goats require about 20 times more plant material than cattle. Acute poisoning is rare and is characterised by sudden death from haemorrhagic liver necrosis and visceral haemorrhages. Chronic exposure is more typical. The signs in horses and cattle include loss of condition, anorexia, dullness and constipation or diarrhea. Residues of alkaloids may appear in milk and other animal products, but these residues are not risky. Another example may be contamination of honey to which the pyrrolizidine alkaloids are transferred by bees. Pyrrolizidine alkaloids have been identified in floral honeys attributed to plants in the Boraginaceae (*Heliotropium* spp., *Echium* spp.) and the Asteraceae (*Senecio* spp.) families. For example, honey from viper's bugloss flowers may contain 0.3–1 mg/kg of alkaloids with echimidine as the main component.

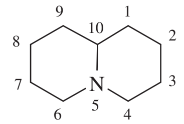
Pyrrolizidine alkaloids are hepatotoxins (substances that deplete the liver) and carcinogens. Characteristic symptoms of toxicose are magnification of liver cells (hepatocytes) called megalocytosis, hyperplasia (enlargement of the liver due to multiplication of cells), liver fibrosis (extensive scarring of the liver) and subsequent symptoms of liver dysfunction, such as hyperbilirubinaemia (an elevated blood level of the pigment bilirubin), hypoalbuminaemia (an abnormally low blood level of albumin), oedema, jaundice and cirrhosis. Alkaloids are not responsible for the toxic effects as such, but rather it is the pyrrole structures (**10-17**) arising from alkaloids that act as alkylating agents. These structures arise by transformation of 1,2-unsaturated compounds (supinidine, retronecine, heliotridine and otonecine derivatives) in the liver. Toxic substances are also aldehydes formed as breakdown product of alkaloids, such as (*E*)-4-hydroxyhex-2-enal.



10-17, toxic metabolites of pyrrolizidine alkaloids

10.3.3.1.4 Quinolizidine alkaloids

Quinolizidine alkaloids, hypothetically derived from quinolizidine (**10-18**), are a special group of bicyclic, tricyclic and tetracyclic secondary metabolites of some legumes, especially legumes of the genera *Lupinus*, *Baptisia*, *Thermopsis*, *Genista*, *Cytisus*, *Chamaecytisus*, *Laburnum* and *Sophora* (Fabaceae), which occur as a complex mixture of several compounds. Quinolizidine alkaloids are also secondary metabolites of some plants of the Chenopodiaceae, Ranunculaceae, Berberidaceae and Solanaceae families.



10-18, quinolizidine

Structure, nomenclature and occurrence

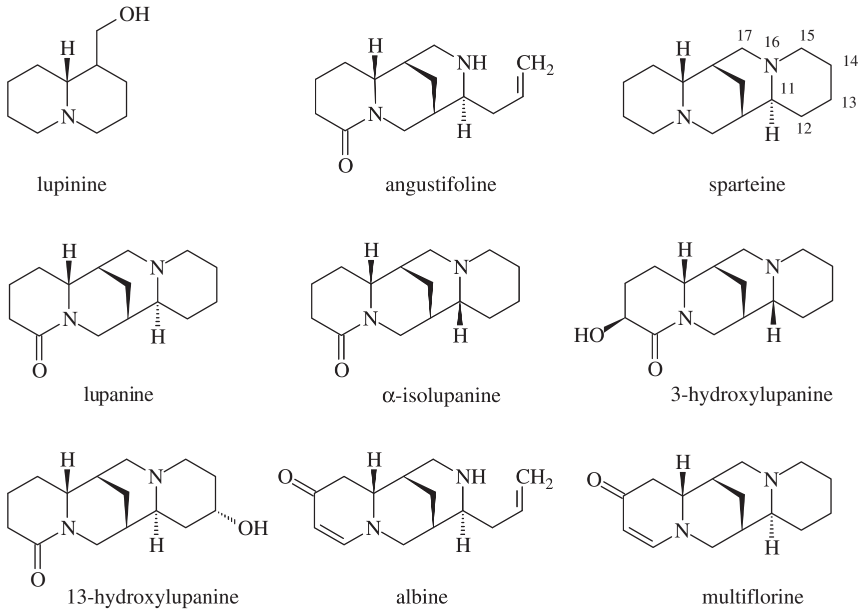
Quinolizidine alkaloids occur in seeds (beans) of certain species of lupines (*Lupinus* spp., Fabaceae), including domesticated varieties. In Europe, Africa and America, lupines have been used as valuable pulses for human nutrition and feeding livestock from ancient times. Native species growing in south-western Europe are white lupine (*L. albus*), blue (narrow leaf) lupine (*L. angustifolius*) and yellow lupine (*L. luteus*). Yellow lupine is currently grown in Western Australia, particularly for feed, and for human nutrition in many countries of Asia. Beans of pearl (Andean) lupine (*L. mutabilis*), called tarhui or tarwi, are eaten as pulses by South American Indians of the Andes region and also used as an oil crop (mainly in Chile).

Seeds of different lupines contain more than 100 bicyclic, tricyclic and tetracyclic quinolizidines. With respect to economically important lupine species, the major alkaloids are lupinine, angustifoline, sparteine, also known as lupinidine, lupanine, α-isolupanine, 3β-hydroxylupanine, 13α-hydroxylupanine, albine and multiflorine (**10-19**). These alkaloids are accompanied by a series of related alkaloids and esters of hydroxylated alkaloids with benzoic, *p*-coumaric, sinapic acids and other organic acids. The hydroxyl group of *p*-coumaric acid is often glycosylated by α-L-rhamnose. The content of alkaloids in plant seeds is highly dependent on the plant species and climatic conditions. The alkaloid content in seeds of original bitter varieties of *L. angustifolius* normally ranges from 1 to 3 and can be up to 5%. The alkaloid content of sweet varieties obtained by breeding is only 0.01–0.03%, but can be also 0.001%. For example, the maximum alkaloid content of seeds of sweet lupine varieties in Australia is 0.002% and in Chile 0.05%.

The composition of the major alkaloids of different lupine seed species is shown in Table 10.3. The main alkaloids of white lupine seeds are lupanine and albine, of blue lupine seeds lupanine and 13-hydroxylupanine and of yellow lupine seeds lupinine and sparteine. The content of other toxic and antinutritional substances (lectins, trypsin inhibitors and phytates) in lupine seeds is similar to the content of other legume seeds.

Reactions and changes

Lupine alkaloids are very stable compounds. During processing and storage of lupine seeds, their content does not change. The most common way of debittering (removal of alkaloids) is extraction of ground seeds with water (by soaking or boiling). These procedures can reduce the alkaloid content by about 100 times.



10-19, quinolizidine alkaloids of lupin seeds

Table 10.3 Main alkaloids content in lupine seeds.

	Content (% of total alkaloid content)			
Alkaloid	***L. albus***	***L. angustifolius***	***L. luteus***[a]	***L. mutabilis***
Lupinine	-	-	60	-
Sparteine	<1	<1	30	16
Albine	15	-	-	-
Angustifoline	<1	10–16	-	1
Lupanine	70	70	<1	46
3-Hydroxylupanine	-	-	-	12
13-Hydroxylupanine	8	12–38	-	7
Multiflorine	3	-	-	-

[a]The seeds of some varieties contain up to 1200 mg/kg of protoalkaloid gramine.

The fat content of lupine is relatively small, except for the South American species *L. mutabilis*, which contains about 22–24% of oil in dry matter). In the technological production of oil from this lupine, about 60% of alkaloids present in bitter varieties are removed during extraction with hexane. Subsequent refining decreases their concentration in the oil down to 0.0005%. Meals are debittered by extraction with ethanol containing hydrochloric acid, which decreases the alkaloid content from the original amount of 3.2% to 0.1–0.2%.

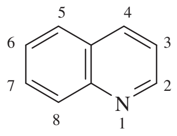
Biological effects

Individual alkaloids exhibit different toxicities as they are hepatotoxic and teratogenic and may represent a hazard for humans and livestock. Lupanine and sparteine are the most toxic substances. Ingestion of 11–25 mg of lupine alkaloids per kilogram of body weight may cause serious health disorders that are manifested by nervousness, vomiting, breathing difficulties, impaired vision and sweating, progressive weakness and, in extreme cases, may end in coma. Alkaloids obtained during lupine seed debittering are used as pesticides and in human medicine.

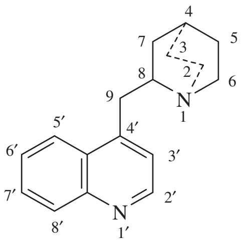
10.3.3.1.5 Quinoline alkaloids

Alkaloids hypothetically derived from quinoline (**10-20**) are a large group of secondary plant metabolites, also called terpenoid indole alkaloids. These alkaloids are found in many plant families, such as Apocynaceae, Loganiaceae, Rubiaceae and Nyssaceae. More than 3000 quinoline alkaloids are known, some of which are used in medicine. The most important quinoline alkaloids are alkaloids

derived from the basic skeleton of rubane (**10-21**), which are found in bark of cinchona or quina trees (*Cinchona* spp., Rubiaceae) native to the tropical forests of western South America, but which today are grown also in other countries.



10-20, quinoline



10-21, rubane

Structure, nomenclature and occurrence

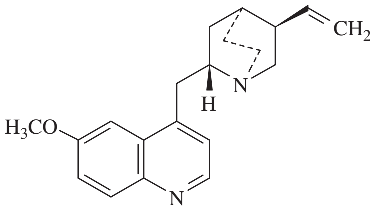
The quinine molecule consists of a quinoline ring with a methoxyl group in position C-6′ and quinuclidine bicyclic structure with a vinyl group (at C-3) in position C-4′, which is bound through carbon C-9 bearing a hydroxyl group. Quinine has asymmetric centres at carbons C-3, C-4, C-8 and C-9. The steric configurations at C-3 and C-4 in the major cinchona bark alkaloids quinine, quinidine, cinchonine and cinchonidine (**10-22**) are the same. Quinidine, the optical isomer of quinine, has a different spatial arrangement of C-8 and C-9 and cinchonidine, which lacks the C-6 methoxyl group, has the same spatial arrangement as quinine. Its isomer is cinchonine, which corresponds in spatial arrangement to quinidine.

The genus *Cinchona* includes about 40 tree species, of which about 12 are of commercial importance. Alkaloids in the bark of *Cinchona officinalis* occur in an amount of 5–8%, of which 2–7.5% is quinine (10-22). The total alkaloid content of *C. succiruba* (syn. *C. pubescens*) bark is 6–16% (quinine content is 4–14%), 5–14% (3–13% represents quinine) is in the bark of *C. ledgeriana* and 3–7%, of which 0–4% is quinine, occurs in *C. calisaya* bark.

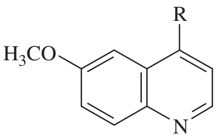
Quinine, isolated from cinchona bark in the form of hydrochloride or sulfate, is mainly used in medicine as an antimalarial and antipyretic medication. In sensory analysis it is used as a standard of bitterness and in the food industry for the production of bitter soft drinks, such as bitter lemon and Indian tonic waters, and in some alcoholic beverages (such as the flavoured wine Barolo Chinato in Italy) for its distinctive bitter taste. The taste threshold concentration of bitter taste perception is about 10 mg/l.

Reactions and changes

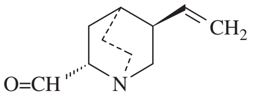
In light, quinine is degraded by photochemical reactions. The main product of degradation in acidic media is 9-deoxyquinine (**10-23**), in slightly acidic and neutral solutions (e.g. in beverages) quinine yields 6-methoxy-4-methylquinoline, 6-methoxyquinoline (**10-24**) and 2-formyl-5-vinylquinuclidine (**10-25**). In drinks exposed to direct sunlight, quinine is totally degraded within 6 hours of exposure, the degradation is accompanied by a light opalescence, and the bitter taste is no longer perceptible. Dark bottles substantially reduce quinine degradation, and artificial light does not affect the quinine stability.



10-23, 9-deoxyquinine



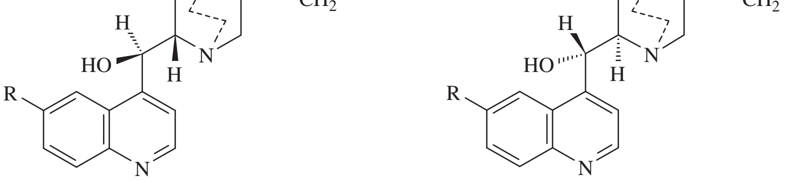
10-24, 6-methoxyquinoline, R = H
6-methoxy-4-methylquinoline, R = CH_3



10-25, 2-formyl-5-vinyl-quinuclidine

Biological effects

Quinine is a protoplasmic toxin (adversely damaging living cells), which inhibits various important enzymes. The ADI value for quinine hydrochloride for adults is 40 mg/kg. Quinine is a teratogenic substance and should never be used by pregnant women, as birth



10-22, cinchona bark alkaloids

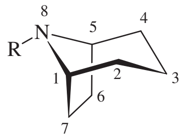
(–)-cinchonidine, R = H
(–)-quinine, R = OCH_3

(+)-cinchonine, R = H
(+)-quinidine, R = OCH_3

defects and miscarriages may occur. Side effects can include ringing in the ears, nausea, blurred vision, chest pain, upset stomachs and breathing problems. The use of quinine is therefore regulated. For non-alcoholic beverages, quinine (sulfate or hydrochloride) may be used in the highest allowable amount of 75 mg/l, while for spirits (bitter alcoholic beverages) the highest amount is 300 mg/l (calculated as free base). According to a new European Commission directive 2002/67/EC, the consumption of quinine in moderation is unlikely to present any health risks and there is no objection from the point of view of toxicology to the continued use of quinine up to a certain maximum level in bitter drinks.

10.3.3.1.6 Calystegines

Calystegines are a relatively new group of bicyclic polyhydroxy alkaloids with a nortropane skeleton (**10-26**), which were originally isolated from the roots of the larger bindweed *Calystegia sepium* (Convolvulaceae). They are biosynthesised together with tropane alkaloids from putrescine via *N*-methylputrescine and other metabolites. The pathway to tropone alkaloids (exemplified, for example, by cocain from the leaves of coca plants, *Erythroxylum coca* and *E. novogranatensen*, Erythroxylaceae, native to western South America) branches and the loss of the *N*-methyl group provides the nortropane skeleton of calystegines.



10-26, nortropane, R = H
tropane, R = CH_3

Structure, nomenclature and occurrence

The structure of calystegines falls into three main classes, calystegines A, B and C (**10-27**), having three, four or five hydroxyl groups, respectively. Diversity within these three classes arises from the positional and stereochemical configuration of these hydroxyl groups. A few calystegines exist that deviate in structure. For example, calystegine N_1 carries an amino group instead of a hydroxyl group on C-1 of the nortropane structure, in addition to three hydroxyl groups on C-2, C-3 and C-4. This calystegine is very labile and undergoes conversion into calystegine B_2. Conversely, calystegine N_1 may be an arefact formed from calystegine B_2 and ammonia.

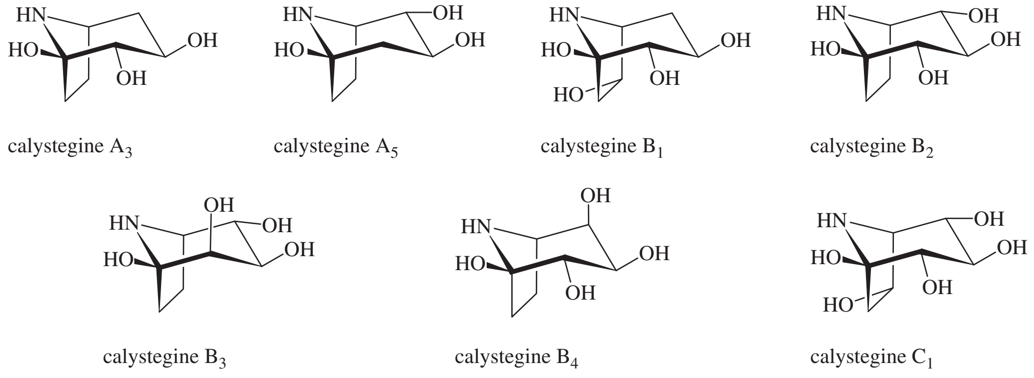
Calystegines have been identified in the plant families Convolvulaceae, Solanaceae, Moraceae and Brassicaceae and have been found in numerous edible fruits and vegetables, especially members of the Solanaceae family, such as potatoes, tomatoes, eggplants and sweet and chili peppers, but also in mulberries and some *Brassica* vegetables and spices, such as kohlrabi, Brussel sprouts and black mustard leaves.

The major calystegines of potatoes are calystegine A_3 and calystegine B_2. Dormant tubers directly after harvest contain less calystegines in all parts than sprouting tubers. In the majority of the potato cultivars their concentration in the peel is between one and two orders of magnitude greater than in the flesh. In the potato cultivars grown in the United States calystegine A_3 and calystegine B_2 ranges were 6–316 mg/kg in dry flesh, 218–2 581 mg/kg in dry peel, 34–326 mg/kg in dry whole potatoes, 1–68 mg/kg in wet flesh, 35–467 mg/kg in wet peel and 5–68 mg/kg in wet whole potatoes. Concentrations in the sproutings are on average 100 times higher than that in the tuber flesh and eight times higher than in the peel. The calystegines in the sproutings include, in addition to the more abundant calystegines A_3 (on average 3999 mg/kg dry matter) and B_2 (7425 mg/kg dry matter), small amounts of four additional types, B_3 (86 mg/kg dry matter), B_4 (319 mg/kg dry matter) and N_1 (15 mg/kg dry matter).

Mulberries (*Morus alba*, Moraceae) contain 4-*O*-β-D-galactopyranosyl-calystegine B_2 (10 mg/kg dry matter), calystegines A_3 and B_2 occur in kohlrabi leaves in amounts of 26 and 7 mg/kg, respectively, calystegine A_3 occurs in the leaves of Brussel sprouts (5 mg/kg dry matter) and calystegines A_3, A_5 and B_2 have been identified in black mustard leaves in concentrations 13, 3 and 2 mg/kg dry matter, respectively.

Reactions and changes

Removal of the peel reduces the calystegine content by an average of over 50% in common potato varieties. Calystegines are water-soluble compounds and their leaching into water can be expected during potato processing.



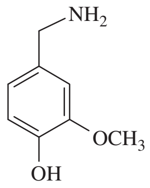
10-27, calystegines

Biological effects

Calystegines exhibit potent specific inhibition of glycosidases that are universally required for normal cell function. Although no human toxicity data for calystegines have been reported, consumption of calystegines has been associated with gastrointestinal upset and it is reasonable to suppose that the calystegines may be responsible, given their propensity to inhibit digestive and hepatic enzymes. The recent discovery of calystegines in widely consumed members of the Brassicaceae family raised concerns about the tolerable overall levels of these alkaloids in the human diet.

10.3.3.1.7 Capsaicinoids

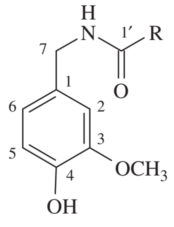
Protoalkaloids capsaicinoids are vanillylamine derivatives, amides of vanillylamine with fatty acids (**10-28**), whose occurrence is restricted to species of hot pepper (*Capsicum* spp.) of the Solanaceae family (see Section 8.3.6.1).



10-28, vanillylamine

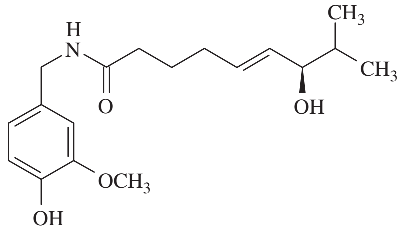
Structure, nomenclature and occurrence

Capsaicinoids (**10-29**) are vanillylamides (*N*-vanillylacylamides) derived from C_8 to C_{11} branched and straight chain *trans*-monoenic and saturated fatty acids. The main components causing a burning sensation when in contact with mucous membranes are capsaicin (*E*)-8-methyl-*N*-vanillylnon-6-enamide and dihydrocapsaicin (8-methyl-*N*-vanillylnonanamide), which constitute about 90% of total capsaicinoids. These capsaicinoids are accompanied by minority alkaloids such as nordihydrocapsaicin, nordihydrocapsaicin II, homodihydrocapsaicin, homodihydrocapsaicin II, homocapsaicin, homocapsaicin II and related compounds, such as pungent (−)-capsaicinol (**10-30**), which also acts as an effective antioxidant.



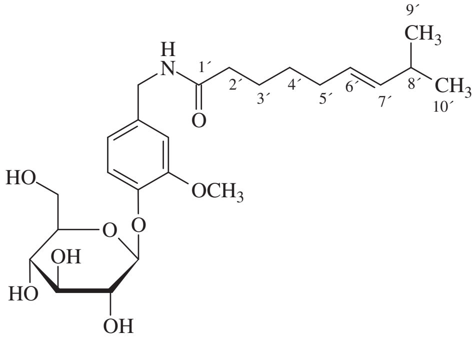
10-29, capsaicinoids

capsaicin, R = $[CH_2]_4CH{=}CHCH(CH_3)_2$
dihydrocapsaicin, R = $[CH_2]_6CH(CH_3)_2$
nordihydrocapsaicin, R = $[CH_2]_5CH(CH_3)_2$
nordihydrocapsaicin II, R = $[CH_2]_4CH(CH_3)CH_2CH_3$
homodihydrocapsaicin, R = $[CH_2]_7CH(CH_3)_2$
homodihydrocapsaicin II, R = $[CH_2]_6CH(CH_3)CH_2CH_3$
homocapsaicin, R = $[CH_2]_4CH{=}CHCH_2CH(CH_3)_2$
homocapsaicin II, R = $[CH_2]_4CH{=}CHCH(CH_3)CH_2CH_3$



10-30, (−)-capsaicinol

The content of capsaicinoids in fruits of *Capsicum* species depends on variety, age, maturity, season and agronomic conditions. Their amount in large bell peppers (*C. annuum*) is usually low, with a higher amount in medium-sized fruits (such as Tabasco, *C. frutescens*) and the highest amount in small chilli peppers (*C. frutescens*). Most alkaloids are found in the flesh and their concentrations are lower in the seeds and skin. Lower amounts of capsaicinoids occur in the young green fruits. The amount of alkaloids increases during the growing and ripening of fruits and reaches a maximum shortly before harvest, and then their concentration decreases slightly. The amount of capsaicinoids in sweeter pepper varieties cultivated in Europe varies in the range of from 0.001 to 0.01%. Chilli peppers typically contain about 0.2–1.5% of alkaloids. For example, of the total amount of 0.4% of capsaicinoids in chilli peppers, about 49% are represented by capsaicin, 44% by dihydrocapsaicin, 6% by nordihydrocapsaicin, 1% by homodihydrocapsaicin and 0.3% by homocapsaicin. In the fruits of hot *C. annuum* cultivars, capsaicin and dihydrocapsaicin can also be found in the form of β-D-glucopyranosides (**10-31**). For example, the concentration of capsaicin-β-D-glucopyranoside ranged from 0.11 to 2.65 mg/kg, while that of capsaicin was 0.03–0.48% fresh weight. Capsaicinoids were also found to be present in vegetative organs, such as stems and leaves. In this case, the proportion of individual capsaicinoids is different to that in fruits, and dihydrocapsaicin is the more abundant compound.



10-31, capsaicin-β-D-glucopyranoside

The burning sensation caused by capsaicinoids in the mouth and throat is their characteristic property. Vanillylamides with a longer or shorter acyl chain than capsaicin have less pungency. There are not particularly large differences between individual capsaicinoids (see Section 8.3.6.1). At concentration of about 10 mg/kg, capsaicin causes burning and pungency, which is perceptible even at a concentration of 0.1 mg/kg. The burning effect is amplified by sucrose and reduced by sodium chloride and solutions with higher viscosity (such as yoghurt). Vanillylamides containing more than 18 carbons in their chain do not stimulate any effect.

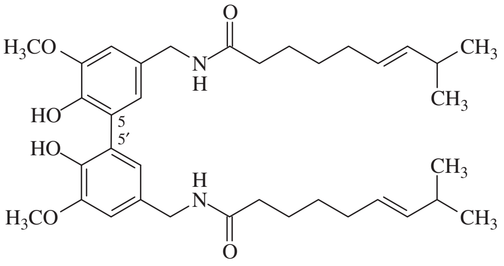
Capsicum plants also contain capsaicinoids with a long-chain acyl moiety. *Capsicum* oleoresin can provide isolation of vanillylamides derived from palmitic acid (palvanil), stearic acid (stevanil), elaidic acid (olvanil) and (9*E*,12*E*)-octadeca-9,12-dienoic acid (livanil) and the existence of myristic acid (myrvanil) and (9*E*,12*E*,15*E*)-octadeca-9,12,15-trienoic acid (linvanil) derivatives was suggested. The content ratios of the total long-chain vanillylamides, except for myrvanil, versus the capsaicin in the oleoresins were significantly larger (0.1–41%) than that in fresh fruits (<0.01%).

Recently identified was another group of non-pungent capsaicinoid analogues produced in *Capsicum* fruits, which were called **capsiconinoids**. Identified compounds were called capsiate, ester of vanillyl alcohol with (*E*)-8-methylnon-6-enoic acid, capsiconiate, ester of coniferyl alcohol with (*E*)-8-methylnon-6-enoic acid and dihydrocapsiconiate, ester of coniferyl alcohol with 8-methylnonanoic acid (**10-32**). The highest content of capsiconinoids was found in *C. baccatum* var. *praetermissum* (3314 mg/kg dry matter) and *C. chinense* (2694 mg/kg dry matter), while their content in *C. annuum* and *C. frutescens* varieties ranged from 0 to 239 and from 0 to 39 mg/kg dry matter.

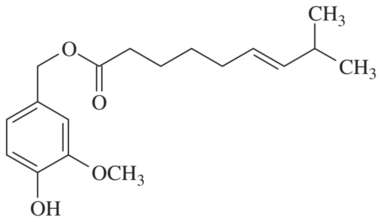
Reactions and changes

Boiling of hot peppers results in a partial leaching of capsaicinoids into water. Their concentration also decreases during other cooking procedures, but in some cases the amount may increase (Table 10.4). Peppers dried in the sun also contain lower amount of capsaicinoids.

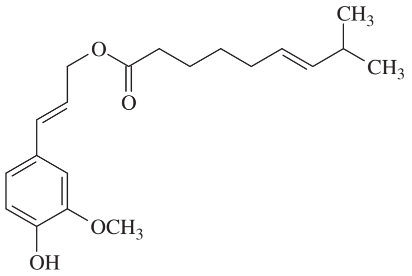
Capsaicinoids are relatively resistant to hydrolysis. Hydrolysis in acidic and alkaline media yields vanillylamine and corresponding fatty acids (their salts). During culinary procedures, which result in tissue disruption, the released enzymes partly hydrolyse capsaicinoids, which are transformed into the higher molecular weight compounds. The action of endogenous peroxidase results, for example, in the formation of 5,5′-dicapsaicin (**10-33**), dimeric 4-*O*-5′-dicapsaicin ether (**10-34**), higher oligomers and copolymers with proteins. In special beverages flavoured with pepper, 5,5′-dicapsaicin may arise by photooxidation in the presence of riboflavin and ascorbic acid (redox system riboflavin/1,5-dihydroriboflavin acts as a photocatalyst).



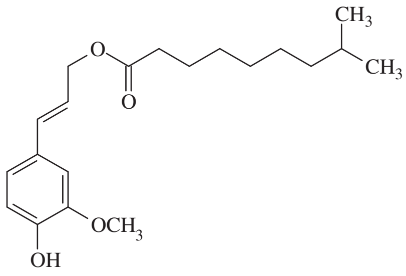
10-33, 5,5′-dicapsaicin



capsiate



capsiconiate

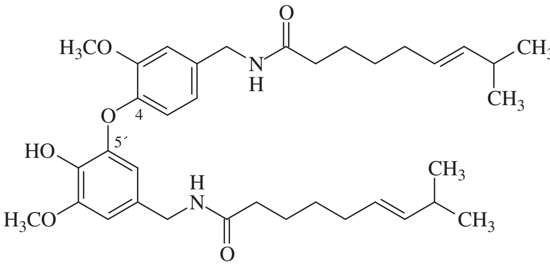


dihydrocapsiconiate

10-32, capsiconinoids

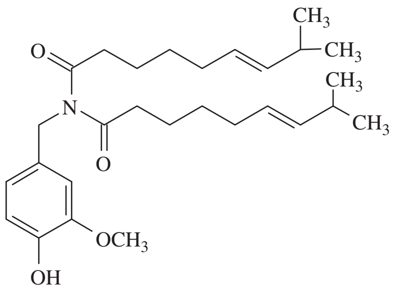
Table 10.4 Influence of technological processing on the content of capsaicinoids in Jalapeño peppers.

	Content (mg/kg)			
Pepper	Capsaicin	Dihydrocapsaicin	Nordihydrocapsaicin	Total
Raw	7300	6300	1200	14 800
Boiled (100 °C, 10 min)	8600	7700	1300	17 000
Blanched and sterilised (100 °C, 50 min)	4700	4500	700	9900
Blanched (100 °C, 3 min) and frozen (−18 °C)	4000	3700	600	8300



10-34, 4-*O*-5′-dicapsaicin ether

Thermal decomposition of capsaicin yields capsaicin dimer (*E*)-*N*-vanillyl-di(8-methylnon-6-en)imide (**10-35**), various amides, acids, hydrocarbons and phenols. The main product is (*E*)-8-methylnon-6-enamide, nonanamide, pentanamide and other amides, (*E*)-8-methylnon-6-enoic acid, nonanoic acid, (*E*)-hept -2-ene, vanillin and other substituted phenols (2-methoxy-4-methylphenol and 2-methoxyphenol), which are produced by decomposition of vanillin. Roasting of peppers in oil may produce fatty acid amides. For example, reaction with oleic acid yields (*Z*)-*N*-vanillyloctadec-9-enamide reaction of oleic acid with ammonia, originating from capsaicin, gives rise to (*Z*)-octadec-9-enamide (oleamide).



10-35, (*E*,*E*)-*N*-vanillyl-di(8-methylnon-6-en)imide

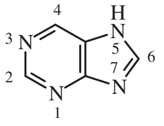
Biological effects

The burning sensation and enhancement of thermogenesis caused by capsaicinoids is induced by the direct activation of a non-selective cation channel, transient receptor potential vanilloid type 1, which is located at the end of sensory nerves. Capsiconinoids have agonist activity for this receptor and they are also involved in promotion of thermogenesis. Capsaicinoids also stimulate intestinal peristalsis and the production of bile, suppress fat accumulation and exhibit weak antimicrobial, antioxidant and anticancerogenic effects. Capsaicinoids derived from long chain fatty acids are anti-inflammatory and antinociceptive substances and enhance adrenaline secretion, despite its lack of irritancy or ungency. Also acting as an antioxidant is (−)-capsaicinol, which has similar effects as α-tocopherol. High concentrations of capsaicinoids may even be toxic.

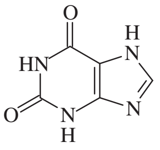
Capsaicin reduces the sensitivity of nerve cells (nocireceptors) for pain and is therefore used in medicine, in creams and sprays, against infectious diseases of the skin and mucous membranes, such as the viral infection *herpes simplex* and psoriasis and seems to be active in the treatment of lung and prostate cancer.

10.3.3.1.8 Purine alkaloids

Alkaloids derived from purine (**10-36**) and purine oxidation product xanthine (**10-37**), respectively, are called purine alkaloids. Purine alkaloids are the most widespread alkaloids in foods.



10-36, purine



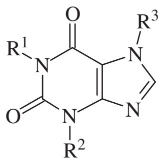
10-37, xanthine

Structure, nomenclature and occurrence

Purine alkaloids are methyl derivatives of xanthine. The most common purine alkaloid is 1,3,5-trimethylxanthine, 1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione, known trivially as caffeine (**10-38**). It is accompanied by dimethylxanthines theobromine, theophylline and paraxanthine and also by methylxanthine heteroxanthine and methyluric acids, which are minority alkaloids, with the exception of cocoa and chocolate. Caffeine is found in the seeds, leaves and fruits of more than 60 species of plants. It is assumed that the purpose of accumulation of purine alkaloids by plants is the protection

Table 10.5 Alkaloids content in green coffee beans.

	Content in dry matter (%)		
Alkaloid	***Coffea arabica***	***Coffea canephora***	***Coffea liberica***
Coffeine	0.53–1.45	2.11–2.72	1.28–1.35
Theobromine	<0.005	<0.005–0.01	<0.005
Theophylline	<0.005	<0.005–0.01	0.01
Trigonelline	0.97–1.31	0.57–0.88	0.25–0.29



10-38, purine alkaloids

caffeine, $R^1 = R^2 = R^3 = CH_3$
theobromine, $R^1 = H$, $R^2 = R^3 = CH_3$
theofylline, $R^1 = R^2 = CH_3$, $R^3 = H$
paraxanthine, $R^1 = R^3 = CH_3$, $R^2 = H$
heteroxanthine, $R^1 = R^2 = H$, $R^3 = CH_3$

of young leaves, flowers and fruits against damage caused by pests. The contents of caffeine, theobromine and theophylline and alkaloid trigonelline in common species of green coffee beans (*Coffea* spp., Rubiaceae) is given in Table 10.5.

The amount of caffeine is dependent on the method of brine preparation (water temperature, time of extraction and other factors). The average content of caffeine in one cup of coffee (100 ml) is about 80 mg, 1–6 mg in decaffeinated coffee, 29–91 mg in instant coffee and 93–127 mg in filtered coffee. Decaffeinated coffee is produced by extraction of caffeine with organic solvents, in particular with dichloromethane or supercritical carbon dioxide. The caffeine obtained is used to enrich soft drinks and in the pharmaceutical industry.

Tea leaves (*Camellia sinensis*, Theaceae) contain about 2% caffeine and less than 0.2% theobromine per dry matter. The mixture of tea alkaloids was previously called **theine**. A normal cup of tea contains about half to one third of the caffeine found in a cup of coffee of the same size.

Leaves of the South American shrub yerba mate or mate plant (*Ilex paraguariensis*, Aquifoliaceae) contain 0.7–2.7% of caffeine per dry matter, between 0.3 and 0.9% of theobromine and traces of theophylline. Mate aqueous extracts have a caffeine content of 61–245 mg/l and theobromine content of 5.2–15.6 mg/l. The mixture of mate alkaloids was previously called **mateine**.

The total alkaloid content of cocoa beans (*Theobroma cacao*, Sterculiaceae) is in the range of from 0.7 to 3.2% of dry matter. The main alkaloid is theobromine, the content of which is 0.6–3.1% and the content of caffeine ranges from 0.02 to 0.5%. Dark chocolate contains 0.3–0.7% of theobromine and 0.02–0.03% of caffeine, theobromine in milk chocolate ranges from 0.1 to 0.4% and the caffeine content is 0.01–0.02%. Chocolate drinks contain theobromine at a level of 260–440 mg/l and 10–12.5 mg/l of caffeine.

In cola drinks (such as Coca Cola and Pepsi-Cola), part of caffeine usually comes from the nuts of some cola species (*Cola acuminata* and *C. nitida*, Malvaceae), where the caffeine content is 1.5–2.5%. The rest is supplemented by caffeine from other sources (it is mainly obtained in the production of decaffeinated coffee). The caffeine content in soft drinks is generally in the range of 50–250 mg/l.

Caffeine occurs at levels of 2.5–7.5% in guarana nuts, seeds of *Paullinia cupana* (Sapindaceae) native to the tropics of South America. These nuts are roasted to a mass that tastes like chocolate. It also serves in the preparation of a refreshing drink. The mixture of guarana alkaloids was previously called **guaranine**.

Reactions and changes

Methylxanthines are very stable compounds and, with the exception of non-enzymatic browning reactions during the fermentation of tea leaves and cocoa beans, they are virtually stable during storage and technological processing of the raw materials. In the manufacture of green and black tea, dimethylxanthines (including theophylline) and other purines result as products of caffeine catabolism.

When roasting coffee, the caffeine content is virtually unchanged. Trigonelline that accompanies coffee alkaloids decomposes to nicotinic acid and volatile sensorially active pyridines, therefore the trigonelline and caffeine contents ratio is used as an indicator of coffee roasting intensity.

Biological effects

Caffeine, together with quinine, is classified as a gustatory and stimulating substance and is used as a food additive. Formerly, its concentration in soft drinks was limited to a highest allowable amount of 250 mg/l; in energy drinks the highest amount was limited to 320 mg/l. The European Commission Directive 2002/67/EC states only that if a beverage intended for consumption contains caffeine in a proportion in excess of 150 mg/l, the product must have on the label message ‘High caffeine content’. In a small daily dose (<3 mg/kg), caffeine acts as a stimulant of the central nervous system and as a diuretic agent. High doses, however, have different neuroendocrine effects, and very high doses are reportedly teratogenic. Theobromine and theophylline exhibit weaker stimulatory effects than caffeine and may cause abnormalities of spermatogenic cells. Theobromine is toxic to some animals, such as dogs, which metabolise theobromine more slowly than humans. Theophylline is used therapeutically in certain respiratory diseases, such as chronic inflammatory disorder of the bronchial tube (bronchial asthma). In sensory studies, the flavanones homoeriodictyol, sterubin and eriodictyol and amides of aromatic amines with hydroxylated benzoic acids (as homoeriodictyol structural analogues) could significantly decrease the bitter taste of caffeine without exhibiting intrinsic strong flavours or taste characteristics.

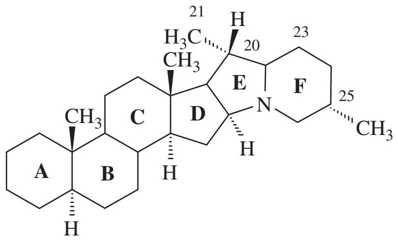
10.3.3.1.9 Steroid glycoalkaloids

Steroid glycoalkaloids (also called steroid alkamines) occur in dicotyledonous plants of the family Solanaceae, Liliaceae and of the subfamily Asclepiadoideae in the Apocynaceae. The fully saturated core of steroid alkaloids is derived from C_{27} hydrocarbon 5α-cholestane, which is composed of three cyclohexane rings (designated as rings A, B and C and one cyclopentane ring (D ring) with a C_8 side chain attached to C-17 in (20*R*)-configuration (see Section 3.7.4.1). Only plants of the genus *Solanum* of the nightshade family, which include potatoes (*S. tuberosum*), eggplants (*S. melongena*) and tomatoes (*S. lycopersicum*), have practical importance in foods.

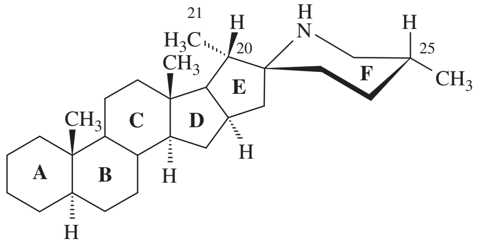
Structure, nomenclature and occurrence

In more than 300 species of plants of the nightshade family, there are at least 90 different steroid glycoalkaloids, which are, according to the structure of the aglycone, divided into five structural types (most of these alkaloids occur as glycosides):

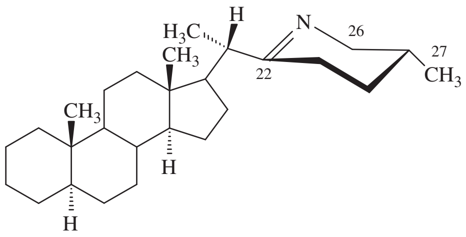
- solanidanes (**10-39**)
- spirosolanes (spiroaminoacetals, **10-40**)
- 22,26-epiminocholestanes (**10-41**)
- α-epiminocyclohemiacetals (**10-42**)
- 3-aminospirostanes (**10-43**).



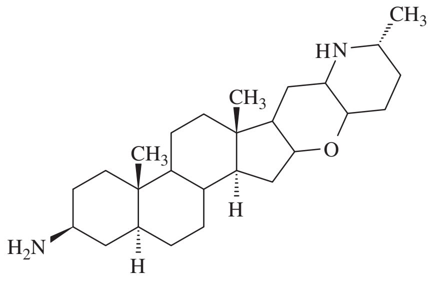
10-39, solanidanes



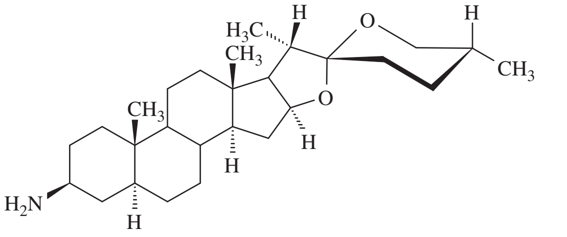
10-40, spirosolanes



10-41, 22,26-epiminocholestanes



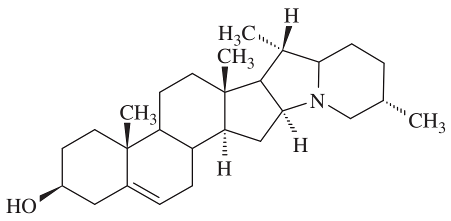
10-42, α-epiminocyclohemiacetals



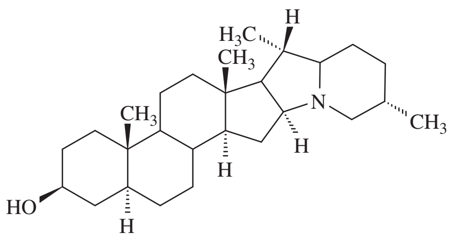
10-43, 3-aminospirostanes

Aglycones

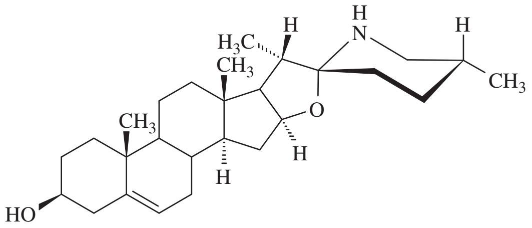
Only glycoalkaloids, the aglycones of which are 3β-hydroxy derivatives of solanidane or spirosolane are found in plant foods used for human nutrition. Solanidane derivatives are solanidine (**10-44**) and demissidine (**10-45**) and solasodine (**10-46**), tomatidenol (**10-47**) and tomatidine (**10-48**) are derived from spirosolane.



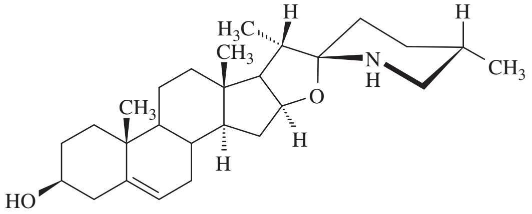
10-44, solanidine



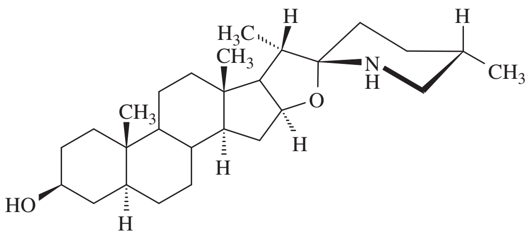
10-45, demissidine



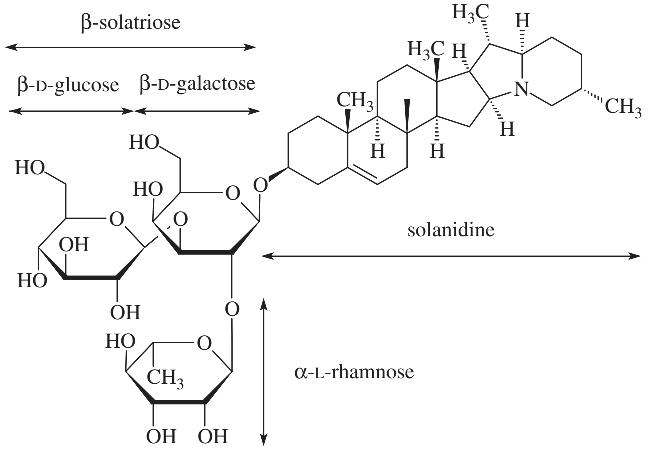
10-46, solasodine



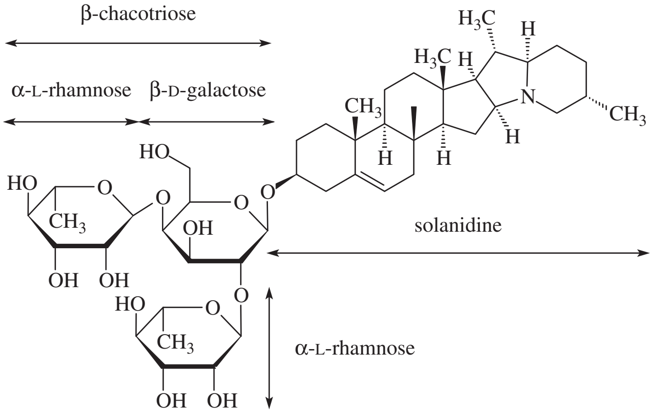
10-47, tomatidenol



10-48, tomatidine



10-49, α-solanine



10-50, α-chaconine

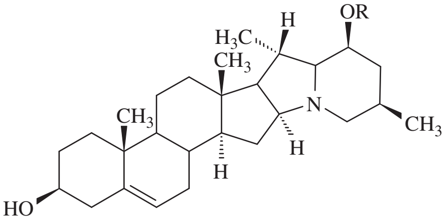
Glycosides

The main compounds in plants are glycosides of sterols, which are accompanied by small amounts of free aglycones. Sugars (linear and branched tetrasaccharides, trisaccharides, disaccharides and monosaccharides) are bound to aglycones via the hydroxyl group at C-3.

Solanidine is the aglycone of two major potato glycoalkaloids that are known as α-solanine (**10-49**) and α-chaconine (**10-50**). Their mixture was previously called solanine. These glycoalkaloids represent about 95% of the potato glycoalkaloids. Sugar bound in α-solanine is β-solatriose; the sugar bound in α-chaconine is β-chacotriose. Besides α-solanine and α-chaconine, cultural potato varieties contain some minority alkaloids, such as β-solanines and β-chaconines, γ-solanine, γ-chaconine, α-solamarine and β-solamarine (Table 10.6). In addition to solanine and chaconine, in the wild potato species growing from the United States to southern Chile there are a number of other glycosides with different aglycones (e.g. leptines, leptinines, commersonine and demissine).

Spirosolanes, namely solasodine, the (25*R*)-stereoisomer, and tomatidenol, the (25*S*)-stereoisomer, are found in wild potato species and varieties obtained by cross breeding the wild varieties, such as *S. berthaultii* and *S. vernei*. They mutually differ only in the position of the heterocyclic nitrogen in ring F. Solasodine occurs as glycoside α-solasonine containing trisaccharide β-solatriose, which is also bound in α-solanine. The same aglycone is in α-solamargine containing β-chacotriose, which is also bound in α-chaconine. Both glycosides, α-solasonine and α-solamargine are found, for example, in wild potato species *S. berthaultii* and *S. vernei* and eggplants (*S. melanogena*).

The (25*S*)-stereoisomer tomatidenol (**10-47**) is present in potatoes in a steroid glycoside α-solamarine containing bound solatriose, or as β-solamarine, which is coupled with β-chacotriose. Most potato varieties containing solamarines come from *S. demissum* species, which also contain (only in the leaves, not in tubers) alkaloids known as leptines and leptinines. The aglycone of leptinines I and II is 23-hydroxysolanidine called leptinidine, the aglycone of leptines I and II is leptidine (23-acetylleptinidine, **10-51**). Leptinine I and leptine I contain β-chacotriose, while leptinine II and leptine II contain β-solatriose (Table 10.6).



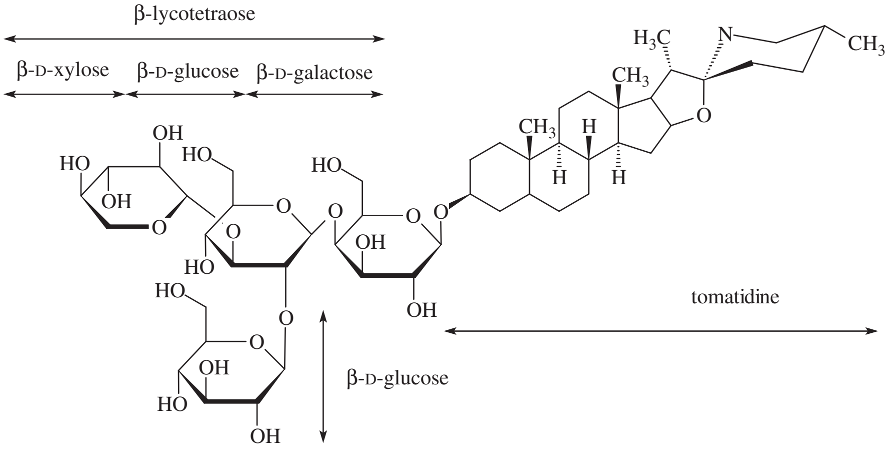
10-51, leptinidine (23-hydroxysolanidine), R = H
leptidine (23-acetylleptinidine), R = $COCH_3$

Table 10.6 Potatoe glycoalkaloids.

Glycoalkaloids	Aglycone	Saccharide	Abbreviated notation
α-Solanine	Solanidine	β-Solatriose	β-D-Glc*p*-(1→3)-[α-L-Rha*p*-(1→2)]-β-D-Gal*p*
β_1-Solanine	Solanidine	β-Neohesperidose	α-L-Rha*p*-(1→2)-β-D-Gal*p*
β_2-Solanine	Solanidine	β-Solabiose	β-D-Glc*p*-(1→3)-β-D-Gal*p*
γ-Solanine	Solanidine	β-D-Galactose	β-D-Gal*p*
α-Chaconine	Solanidine	β-Chacotriose	α-L-Rha*p*-(1→4)-[α-L-Rha*p*-(1→2)]-β-D-Glc*p*
β_1-Chaconine	Solanidine	β-Neohesperidose	α-L-Rha*p*-(1→2)-β-D-Glc*p*
β_2-Chaconine	Solanidine	β-Chacobiose	α-L-Rha*p*-(1→4)-β-D-Glc*p*
γ-Chaconine	Solanidine	β-D-Glucose	β-D-Glc*p*
α-Solasonine	Solasodine	β-Solatriose	β-D-Glc*p*-(1→3)-[α-L-Rha*p*-(1→2)]-β-D-Gal*p*
α-Solamargine	Solasodine	β-Chacotriose	α-L-Rha*p*-(1→4)-[α-L-Rha*p*-(1→2)]-β-D-Glc*p*
α-Solamarine	Tomatidenol	β-Solatriose	β-D-Glc*p*-(1→3)-[α-L-Rha*p*-(1→2)]-β-D-Gal*p*
β-Solamarine	Tomatidenol	β-Chacotriose	α-L-Rha*p*-(1→4)-[α-L-Rha*p*-(1→2)]-β-D-Glc*p*
α-Tomatine	Tomatidine	β-Lycotetraose	β-D-Xyl*p*-(1→3)-[β-D-Glc*p*-(1→2)]-β-D-Glc*p*-(1→4)-β-D-Gal*p*
Demissine	Demissidine	β-Lycotetraose	β-D-Xyl*p*-(1→3)-[β-D-Glc*p*-(1→2)]-β-D-Glc*p*-(1→4)-β-D-Gal*p*
Commersonine	Demissidine	β-Commertetraose	β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→2)]-β-D-Glc*p*-(1→4)-β-D-Gal*p*
Leptinine I	Leptinidine	β-Chacotriose	α-L-Rha*p*-(1→4)-[α-L-Rha*p*-(1→2)]-β-D-Glc*p*
Leptinine II	Leptinidine	β-Solatriose	β-D-Glc*p*-(1→3)-[α-L-Rha*p*-(1→2)]-β-D-Gal*p*
Leptine I	23-Acetylleptinidine	β-Chacotriose	α-L-Rha*p*-(1→4)-[α-L-Rha*p*-(1→2)]-β-D-Glc*p*
Leptine II	23-Acetylleptinidine	β-Solatriose	β-D-Glc*p*-(1→3)-[α-L-Rha*p*-(1→2)]-β-D-Gal*p*

The β-glycoside of tomatidenol in tomatoes, called dehydrotomatine, accompanies α-tomatine in tomatoes. Dehydrotomatine and α-tomatine contain bound lycotetraose. The (25*S*)-stereoisomer tomatidine (**10-48**), also known as 5,6-dihydrotomatidenol, is an aglycone of the main tomato glycoalkaloid α-tomatine (**10-52**). α-Tomatine also occurs in small amounts in some potato species and some other species of the genus *Solanum*. In the wild potatoes (*S. acaule*) its content ranges from to 74 to 497 mg/kg fresh weight.

The distribution of glycoalkaloids differs in the various parts of plants. α-Solanine and α-chaconine occur in roughly equal amounts. The largest amount of solanine in potato is present in the flowers (5000 mg/kg) and germ (1950–4360 mg/kg). The highest concentrations in tubers are in the surface layers. Towards the



10-52, α-tomatine

Table 10.7 Distribution and content of glycoalkaloids in potatoes.

Part of tuber	Content (mg/kg) (fresh weight)
Unpeeled	75
Peel (2-3% of tuber weight)	300-600
Peel (10-15% of tuber weight)	150-300
Peel with germ (3 mm)	300-500
Peeled	12-50

centre of the tubers, the solanine content decreases (Table 10.7). Higher amounts of solanine generally contain small tubers. Usually, the amount of solanine in potato tubers does not exceed 200 mg/kg, except for the wild species. Potatoes containing more than 140 mg/kg of glycoalkaloids exhibit bitter taste, potatoes containing more than 220 mg/kg have a hot taste. The amount of alkaloids is strongly dependent on soil and climatic conditions. In tubers exposed to light and in injured tubers the alkaloid biosynthesis increases by up to 400%. The stimulating effect mainly comes from light of shorter wavelengths; the light of longer wavelengths primarily stimulates the biosynthesis of chlorophyll.

Tomatine is practically the only glycoalkaloid of tomatoes. Tomatine is accompanied in small amounts by glycoalkaloids containing as sugar components the products of partial hydrolysis of lycotetraose (β_1-tomatine with trisaccharide produced by hydrolysis of xylose from lycotetraose, β_2-tomatine with trisaccharide without glucose and γ-tomatine with disaccharide without xylose and glucose). Tomatine is present in all parts of the plant, with the highest amount located in the leaves, flowers and small green fruits. The biosynthesis of tomatine in fruits is temporary and during their maturation the tomatine content in fruits decreases. Tomatine concentrations in mature red fruits are very small (Table 10.8).

The amount of α-solasonine and α-solamargine in various species, hybrids and varieties of eggplants ranged from 36 to 691 mg/kg dry matter and from 140 to 7700 mg/kg dry matter, respectively, with current eggplant genotypes in the range of 92–125 mg/kg dry matter and 229–299 mg/kg dry matter, respectively.

Table 10.8 Tomatine content in tomatoes. Kozukue, Han, Lee and Friedman, 2004, table 1. Reproduced by permission of the American Chemical Society.

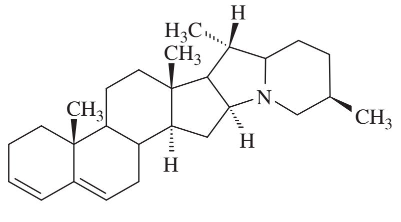
State of ripeness (days after flowering)	Content (mg/kg) (fresh weight)	
	Tomatine	Dehydrotomatine
Small and green (10)	795	60
Large and green (20)	49	2.6
Pink (30)	20	trace
Light red (40)	1.5	0
Red and ripe (50)	3.7	0

Reactions and changes

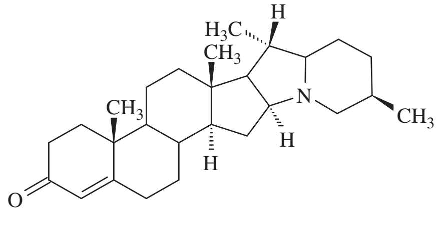
Alkaloids in potatoes are mainly removed by peeling (60–90%), but they are relatively stable during technological and culinary processing. The degradation of alkaloids does not occur even during the production of potato crisps and French fries, but their relative content increases due to loss of water during frying. When cooking potatoes, some loss of alkaloids occurs due to leaching into water. Adding acetic acid can increase this loss. The addition of 0.3% of acetic acid reduces the solanine content in cooked potatoes by about 84%.

Opinions on changes of alkaloids during potato storage vary. Some studies have shown an increase in alkaloid content at lower temperatures. Higher temperature and lower relative humidity are the optimal conditions for preventing the formation of glycoalkaloids during potato storage.

The activity of hydrolases or hydrolysis in acidic media leads to the hydrolysis of glycoalkaloids, but the released aglycones are practically stable. For example, α-chaconine in germinating potatoes is gradually hydrolysed to β_2-chaconine, but the α-solanine content is unchanged. Partial acid hydrolysis of α-chaconine and α-solanine leads gradually to approximately equimolar quantities of β_1-glycosides, β_2-glycosides and γ-glycosides and the final product is aglycone solanidine. The same products may also arise in the digestive tract. Upon heating in mineral acids, solanidine partially dehydrates to solanthrene (**10-53**) and other minor products, such as the oxidation product solanid-4-en-3-one (**10-54**). These reactions can be expected to a lesser extent during thermal processing of potatoes.



10-53, solanthrene



10-54, solanid-4-en-3-one

Tomatine is relatively stable under normal storage and processing conditions. In acid media partial hydrolysis of lycotetraose can be expected. Freezing of unripe tomatoes (unpeeled, green–yellow tomatoes) results in approximately 8% loss of tomatine, freezing of peeled tomatoes leads to an 18% loss of alkaloids, which may be attributed to tissue damage and action of hydrolytic enzymes.

Biological effects

Levels of α-solanine and α-chaconine normally found in potatoes (20–100 mg/kg) are not of toxicological concern. Typical manifestations of higher levels, the so-called solanine poisoning, are vomiting, diarrhea, stomach cramps, headaches and dizziness. α-Solanine toxicity is attributable either to the inhibition of cholinesterase or damage to the digestive tract membranes and some organs. An even more effective inhibitor of cholinesterase is α-chaconine. High doses of α-chaconine have teratogenic effects. In some countries, and formerly also in the EU, the permissible concentrations of α-solanine and α-chaconine were set to 200 mg/kg.

Tomatine has antifungal and teratogenic effects. Toxic effects are often associated with the ability of 3β-hydroxysterols to bind tomatine in membranes, which destabilises the lipid bilayer. For tomato alkaloids (α-solanine, α-chaconine and α-tomatine) the same maximum amount has therefore been set as for potato alkaloids.

10.3.3.2 Saponins

10.3.3.2.1 Structure, nomenclature and occurrence

Saponins are a diverse group of surface-active heteroglycosides occurring mainly in plants. Hydrophobic aglycones of saponins, which are called sapogenols (formerly sapogenins) are compounds derived from:

- C_{30} triterpenoids, also known as triterpenic alcohols
- C_{27} steroids.

The aglycone is bound to one or more sugar residues. In **monodesmosides**, one sugar residue (mono- or oligosaccharide) is bound to the aglycone (sugar is normally bound to the C-3 hydroxyl), **bisdesmosides** have sugar residues bound in different positions and three sugar residues bound in different positions are found in **trisdesmosides**. Common sugars are usually L-arabinose, D-glucose, D-mannose, D-galactose, L-rhamnose, D-glucuronic acid and D-galacturonic acid, less frequently D-xylose and D-apiose, but some other sugars may also be present. Sugars can also be acylated with organic acids, such as acetic acid. The amount of saponins depends mainly on the plant species and climatic conditions. The largest concentration of saponins is located in the roots, bark and fast-growing parts of the plant (Table 10.9). At low concentrations, saponins are also present in certain marine organisms and bacteria.

The physiological role of saponins in plants is not yet fully understood; nevertheless it is assumed that they represent a part of plants' defence systems of protective molecules named phytoprotectants, chemical agents that interacts with a pathogen on the plant surface to prevent infection, either phytoanticipins or phytoalexins (see Section 10.3.3.6).

Triterpenoid saponins

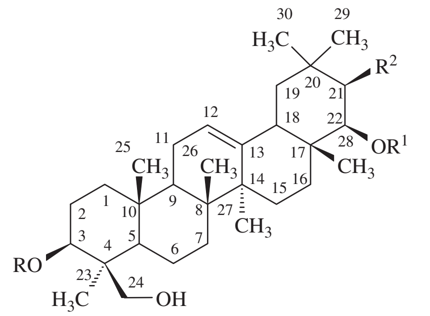
Triterpenoid saponins are common components of many plants. Their structure is mainly derived from pentacyclic triterpenoids, such as lupeol, α-amyrine and β-amyrine (see Section 3.7.4.1.1). In positions C-4 (C-23 methyl), C-17 (C-28 methyl) and C-20 (C-30 methyl) of the aglycone system C_6–C_6–C_6–C_6–C_6 a carboxyl group formed by oxidation of a methyl group may occur. Methyl groups of some saponins are only partially oxidised to hydroxymethyl and formyl groups and some aglycones contain other hydroxyl groups at C-21 and C-22. Positions C-11 and C-16 may also be oxidised. Sugar moieties (1–6) are bound to the C-3 hydroxyl and some hydroxyl groups may be esterified with carboxylic acids, for example with acetic acid (C-22 hydroxyl) or angelic acid (C-21 hydroxyl).

The best known saponins are soyasaponins, aglycones of which are soyasapogenol A (oleane-12-en-3β,22β,24-triol), soyasapogenol B (olean-12-en-3β,22β,24-triol, **10-55**), soyasapogenol E (oleane-12-en-3β,24-diol-22-one, **10-56**), hederagenin (**10-57**), medicagenic acid (**10-58**) and bayogenin (**10-59**). The content of saponins in seeds of the soybean ranges from 0.6% to as much as 6.5% dry matter, depending on the variety, cultivation year, location grown and degree of maturity.

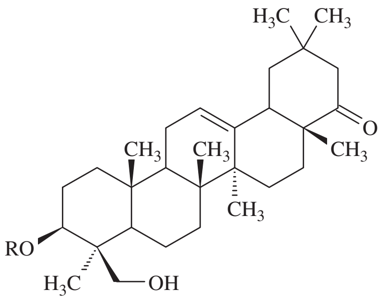
Table 10.9 Saponins content in legumes and other plants.

Plant	Latin name	Content (%)	Plant	Latin name	Content (%)
Fabaceae			Lentil	*Lens culinaris*	0.11–0,51
Soya bean	*Glycine max*	0.22–5.6	Peanut	*Arachis hypogaea*	0.01–1.6
Common bean	*Phaseolus vulgaris*	0.35–1.6	Liquorice	*Glycyrrhiza glabra*	2.2–15.0
Lima bean[a]	*Phaseolus lunatus*	0.10	***Amaranthaceae***[c]		
Mung bean[b]	*Vigna radiata*	0.34	Spinach	*Spinacia oleracea*	4.7
Chickpea	*Cicer arietinum*	0.23–6.0	Beet	*Beta vulgaris*	5.8
Pea	*Pisum sativum*	0.11–0.18	Quinoa	*Chenopodium quinoa*	0.14–2.3

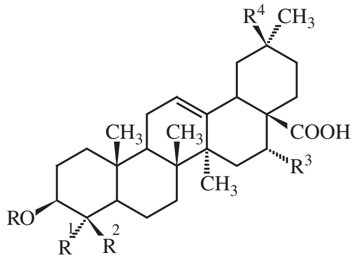
[a] Also known as butter bean.
[b] Also known as green gram.
[c] Formerly Chenopodiaceae.



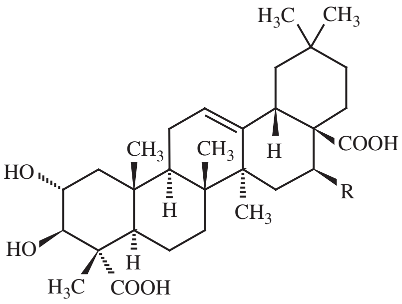
10-55, soyasapogenol A, R = H, R^1 = H, R^2 = OH
soyasapogenol B, R = H, R^1 = H, R^2 = H
soyasaponin A_a, R=β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→, R^1= β-D-Xyl*p*2,3,4Ac_3-(1→3)-α-L-Ara*f*-(1→, R^2= OH
soyasaponin A_b, R=β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→, R^1=β-D-Glc*p*2,3,4,6Ac_4-(1→3)- α-L-Ara*f*-(1→, R^2= OH
soyasaponin B_a, R=β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→, R^1 = H, R^2 = H
soyasaponin B_b, R=α-L-Rha*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→, R^1 = H, R^2 = H



10-56, soyasapogenol E, R = H
soyasaponin B_d, R = β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→
soyasaponin B_e, R = α-L-Rha*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→

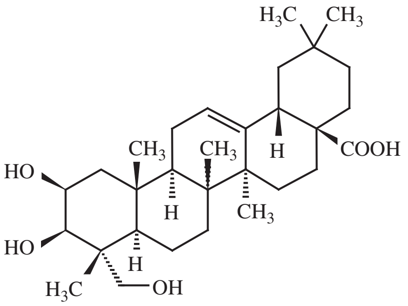


10-57, oleanolic acid, R = R^1 = R^2 = R^3 = R^4 = H
hederagenin, R = H, R^1= CH_2OH, R^2 = R^3 = R^4 = H
phytolaccagenic acid, R = H, R_1= CH_2OH, R_2 = R_3 = H, R^4 = $COOCH_3$
quillajic acid, R = H, R^1= CH=O, R^2 = H, R^3 = OH, R^4 = H
gypsogenic acid, R = H, R^1= CH=O, R^2 = R^3 = R^4 = H



10-58, medicagenic acid, R = H
zanhic acid, R = OH

Saponins derived from soyasapogenol A are bisdesmosides containing sugars bound to C-3 and C-22. Some of the sugars can be acetylated. Acetylated derivatives have a bitter and astringent taste. Most varieties of soya contain soyasaponin A_a and A_b (**10-55**) as the main representative of the acetylated compounds. Glycosides of soyasapogenol B, such as soyasaponin B_a and soyasaponin B_b, still often referred to as soyasaponin I (**10-55**), lack the C-22 hydroxyl group; they belong to monodesmosides, and the sugar moiety is attached to C-3 hydroxyl. Additional soyasaponins are derived from soyasapogenol E, for example, saponin B_d and saponin B_e (**10-56**). A recently identified group of soyasaponins B_aA and B_bA (also known as soyasaponin βg, soyasaponin VI or chromosaponin I) are conjugates of soyasaponin B_b with γ-pyrone (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one, which is bound to carbon

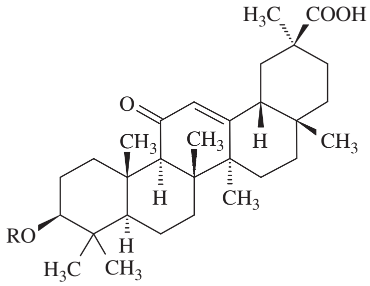


10-59, bayogenin

C-22 (**10-60**). These saponins are thus actually bisdesmosides. Soyasaponin B_b and its conjugates with γ-pyrane occur as the main saponins in some other legumes, for example, in common beans and lima beans, pea, lentils and chickpeas.

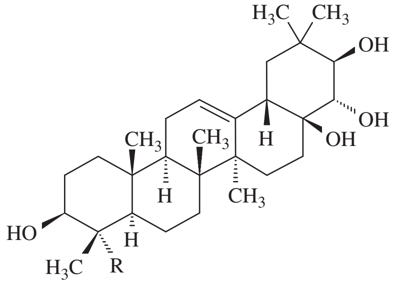
Plants of the Amaranthaceae (formerly Chenopodiaceae) family, for example sugar beet (*Beta vulgaris* group *Altissima*), contain about 5.8% saponins. The main sapogenin is oleanolic acid. Spinach contains about 4.7% saponins, derived from oleanolic acid and hederagenin (**10-57**). The edible grain quinoa of the same plant family (*Chenopodium quinoa*), originating in South America, contains as aglycones phytolaccagenic acid (**10-57**), hederagenin and oleanolic acid (**10-57**). Aglycones of soap tree bark (*Quillaja saponaria*, Quillajaceae) saponins, which are used industrially for pharmaceutical and cosmetic products, are guillajic and gypsogenic acids (**10-57**). Their content in soap tree bark is around 10%. Similar structures are seen in medicagenic (**10-58**) and zanhic acids (**10-58**), which are bis- and trisdesmosides, respectively, found in alfalfa (*Medicago sativa*, Fabaceae) together with saponins, whose aglycone is soyasapogenol B. The main saponin is trisdesmoside of zanhic acid, where R = β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→, R^1=α-L-Ara*p*-(1→, R^2=β-D-Api*f*-(1→3)-β-D-Xyl*p*-(1→4)-α-L-Rha*p*-(1→2)-α-L-Ara*p*-(1→. Related bayogenin (**10-59**) occurs in soya beans.

The rhizome of liquorice (*Glycyrrhiza glabra*) of the same plant family contains 2.2–15.0% of saponins and has an intensely sweet taste. It is used in the production of various pharmaceutical and confectionery products. The primary representative of liquorice saponins is glycyrrhizin, which is a mixture of potassium and calcium salts of monodesmoside known as glycyrrhizic acid. The glycyrrhizin aglycone is glycyrrhetic (glycyrrhetinic) acid (**10-61**), to which are bound two β-D-glucuronic acid units. The sweetness of glycyrrhizin is between 50 and 150 times higher than the sweetness of sucrose.



10-61, glycyrrhetic acid, R = H
glycyrrhizic acid, R = β-D-Glc*p*A-(1→2)-β-D-Glc*p*A-(1→

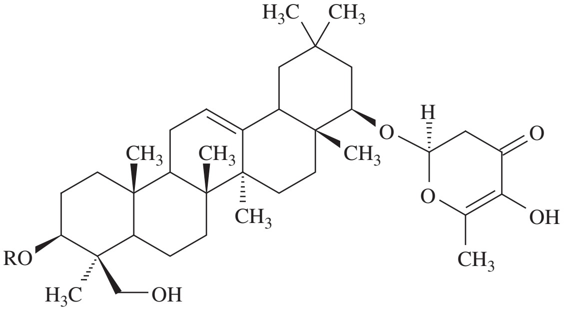
Saponins of tea leaves (*Camellia sinensis*, Theaceae) contain as the main components theasapogenol A and theasapogenol B (**10-62**). Other related compounds include theasapogenols C, D and E, assamsapogenols (A, B, C and D).



10-62, theasapogenol A, R = CH_2OH
theasapogenol B, R = CH=O

Steroid saponins

Saponins that have in the molecule steroids as aglycones (steroid saponins) contain aglycones based on C_{27} structures of spirostanol or furostanol formed by modifications of the cholesterol side chain.

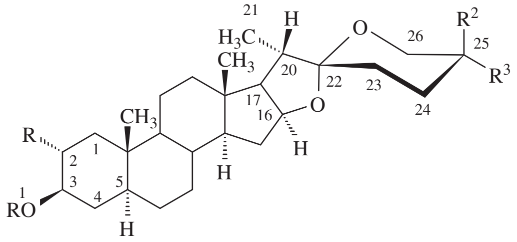


10-60, soyasaponin B_aA, R = β-D-Glc*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→,
soyasaponin B_bA, R = α-L-Rha*p*-(1→2)-β-D-Gal*p*-(1→2)-β-D-Glc*p*A-(1→

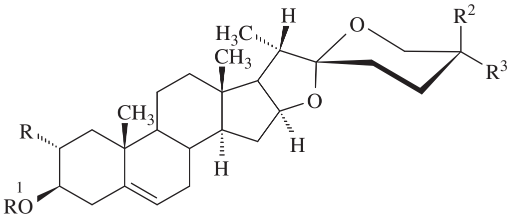
Sugars (2–5 residues of hexoses or pentoses) are attached to the C-3 hydroxyl group of aglycones. Other conjugates of this type also include other triterpenes with sugars attached at different positions.

Steroidal saponins are less widespread in comparison with triterpenoid saponins. They typically occur in monocotyledonous plants of the families Agavaceae, Amaryllidaceae, Asparagaceae, Dioscoreaceae and Poaceae, but are also present in some dicotyledonous plants, for example in legumes of the family Fabaceae. Both types of saponins are often present in the same plant material.

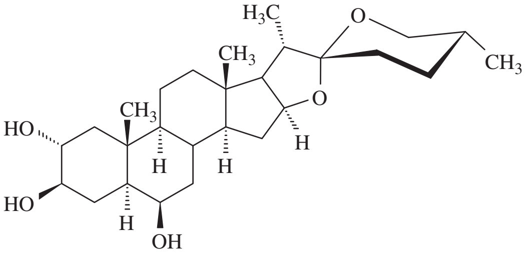
Plants of the Allioideae subfamily (the old Alliaceae family), for example, onion (*Allium cepa*), contain saponins derived from oleanolic acid, β-amyrin (see **3-99**), gitogenin (**10-63**), diosgenin (**10-64**), β-chlorogenin (**10-65**) and cepagenin (**10-66**). Recently identified were spirostane-type saponins with trivial names alliospirosides A to D (**10-67**), tropeosides A1, A2, B1, B2 and ascalonicosides A1, A2, B. For example, the sugar bound in allispiroside A is a disaccharide α-L-Ara*f*-(1→2)-6-deoxy-α-L-Man*p*-, tropeoside A1 (**10-68**) contains two monosaccharides,



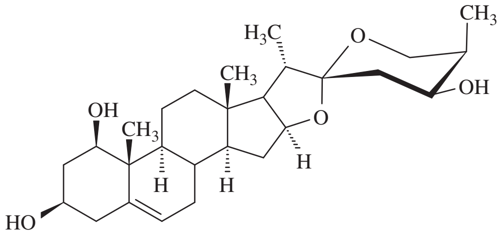
10-63, tigogenin, R = H, R^1 = H, R^2 = CH_3, R^3 = H
neotigogenin, R = H, R^1 = H, R^2 = H, R^3 = CH_3
gitogenin, R = OH, R^1 = H, R^2 = CH_3, R^3 = H
neogitogenin, R = OH, R^1 = H, R^2 = H, R^3 = CH_3



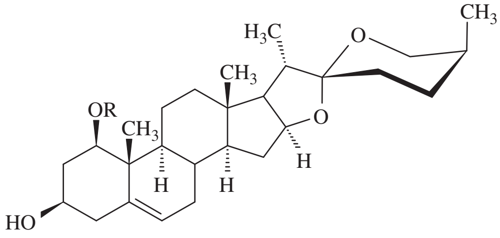
10-64, diosgenin, R = H, R^1 = H, R^2 = CH_3, R^3 = H
yamogenin, R = H, R^1 = H, R^2 = H, R^3 = CH_3
yuccagenin, R = OH, R^1 = H, R^2 = CH_3, R^3 = H
lilagenin, R = OH, R^1 = H, R^2 = H, R^3 = CH_3



10-65, β-chlorogenin

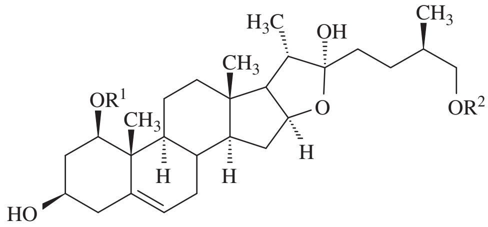


10-66, cepagenin



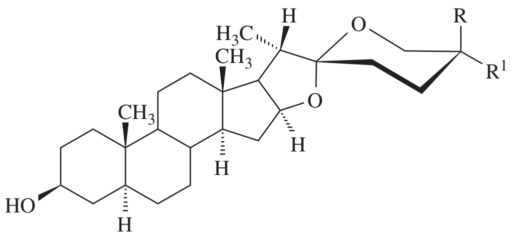
10-67, alliospiroside A

R^1 = β-D-Gal*p*, R^2 = α-L-Rha*p*, and ascalonicoside A1 (**10-68**) contains one monosaccharide (R^1 = β-D-Gal*p*) and one disaccharide (R^2 = α-L-Rha*p*-(1→2)-β-D-Glc*p*-). Saponins of garlic (*Allium sativum*) are derived from phytosterol sitosterol (see **3-119**) and saponins of leek (*Allium ampeloprasum*) contain oleanolic acid and gitogenin (**10-63**) as aglycones.



10-68, tropeoside A1/ascalonicoside A1

Saponins of asparagus (*Asparagus officinalis*, Asparagaceae) are trivially called officinalisins and asparagosins. For example, officinalisins contain 5β-furostan-3β,22α,26-triol as aglycone, aglycone of asparagosins is (25*S*)-spirost-5-en-3β-ol trivially called yamogenin (**10-64**). Saponins are located in larger quantities at the bottom of the stalks, to which they give a bitter taste. Present as a minor aglycone is sarsapogenin derived from spirostane (**10-69**).



10-69, smilagenin, R = CH_3, R^1 = H
sarsapogenin, R = H, R^1 = CH_3

At least nine compounds known as asparagosides (asparagosides A to I) have been characterised.

Yamogenin and yamogenin isomer diosgenin, (25*R*)-spirost-5-en-3β-ol (**10-64**) are the aglycones of saponins in yams (*Dioscorea* spp., Dioscoraceae) whose starchy tubers are eaten in Africa, South-east Asia and the Pacific region (*D. alata* and *D. esculenta* in South-east Asia, *D. trifida* in South America, *D. rotundata* and *D. cayenensis* in West Africa). Yams typically contain 4–8% saponins, which are also a source of steroids for the pharmaceutical industry.

A number of steroid saponins called fenugrins and grecunins are found in the legume plant fenugreek (*Trigonella foenum-graecum*, Fabaceae), which is used as a spice and fodder plant. In India, for example, fenugreek leaves are eaten as a salad and in European countries the seeds are used as a spice. The main aglycone of the seeds is diosgenin; in smaller quantities are present yamogenin (**10-64**), gitogenin, neotigogenin (**10-63**), smilagenin, sarsapogenin (**10-69**) and their 3α-epimers, known as epismilagenin and episarsapogenin. Minor fenugreek components are dihydroxy derivatives yuccagenin, lilagenin (**10-64**), gitogenin and neogitogenin (**10-63**).

A similar structure is seen in steroid bisdesmosides of oats (*Avena sativa*, Poaceae), which are called avenacosides (**10-70**). Specific β-glucosidase present in oat leaves hydrolyses avenacosides (splits-off glucose bound at C-26) to monodesmosides called deglucoavenacosides, which are less bitter, but exhibit higher antimicrobial and haemolytic effects.

10.3.3.2.2 Reactions and changes

Saponins are relatively stable during common technological and culinary operations. Their amount can be reduced by adequate washing, maceration or removing the surface layer (peeling). Sugar beet saponins are removed during the sugar refining, soya bean saponins are removed by debittering using various technological processes, such as peeling, acid hydrolysis and fermentation processes (using suitable microorganisms such as moulds *Aspergillus oryzae* and *A. niger*), in which bitter saponins are enzymatically hydrolysed to non-bitter aglycones.

The content of saponins may be decreased by dipping in water and cooking (or sterilisation). When cooking legumes, bitter glycosides are partly dissolved and partly hydrolysed to aglycones and sugars. For example, losses of saponins during cooking lentils may reach 6–14% of the original amount, which is about 700–1100 mg/kg dry weight. The highest reduction of saponins in legumes can be achieved by soaking, followed by germination for about 40 hours and boiling. Under these circumstances, the content of saponins may be reduced by up to 75%.

Triterpenic saponins derived from soyasapogenol E (B_d and B_e saponins) are highly unstable and during processing of legumes, they are reduced to B_a and B_b saponins, respectively. During the thermal processing of legumes, soyasaponin B_eA is partially degraded to soyasaponins B_b and B_e, which yields the respective aglycone soyasapogenol E. Glucose at position C-26 of steroid furostane type saponins can be split-off by enzymatic hydrolysis or the activity of microorganisms, and furostane derivatives are transformed into spirostane derivatives (Figure 10.2). Similarly to the case of sterols, also saponin aglycones may partially eliminate the hydroxyl group at C-3 as water, with the formation of corresponding unsaturated hydrocarbons.

10.3.3.2.3 Biological effects

In the past, practically all saponins were considered antinutritional or toxic substances as they negatively influence organoleptic properties of food causing unwanted bitterness and astringency in soybeans and other legumes and also animal nutritionists have generally considered saponins to be deleterious compounds that negatively affect growth, feed intake and reproduction in animals.

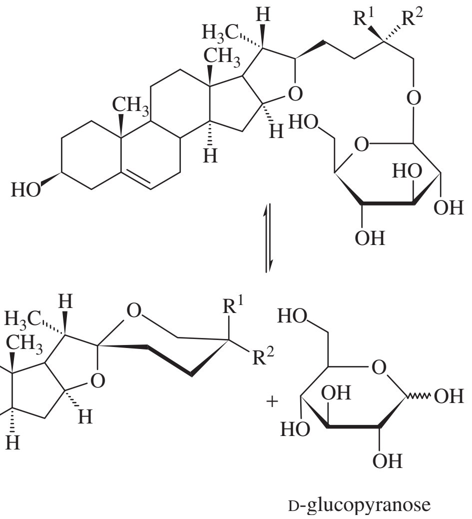
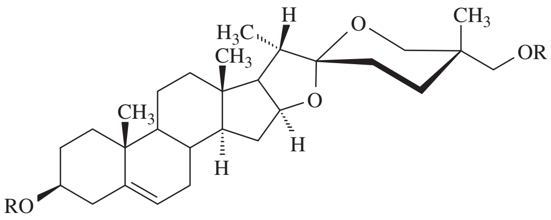


Figure 10.2 Interconversion of furostane and spirostane structures.



10-70, avenacoside A, R =β-D-Glc*p*-(1→2)-[α-L-Rha*p*-(1→4)]-β-D-Glc*p*-(1→, R^1=β-D-Glc*p*-(1→
avenacoside B, R=β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-[α-L-Rha*p*-(1→4)]-β-D-Glc*p*-(1→, R^1=β-D-Glc*p*-(1→

Saponins are also highly toxic to cold-blooded organisms (such as insects and fish). Toxic saponins are often called **sapotoxins**. Their toxic effect is manifested by haemolysis of erythrocyte cells and damage of intestinal mucosa. The main reason is the interaction of saponins with cholesterol in the cell walls. High doses of toxic saponins may damage the liver, which can lead to respiratory failure. In retrospect, however, only some saponins are really toxic.

Analogously as with cholesterol, saponins react with other sterols and bile acids (under micelle formation) and thereby inhibit their absorption, which is related to the metabolism of cholesterol and prevention of cardiovascular diseases. Recently, a number of studies have reported both beneficial and adverse effects of these compounds in a variety of animals. Extensive research carried out into the membrane-permeabilising, immunostimulant, hypocholesterolaemic, hypoglycaemic, antioxidative (e.g. conjugates of saponin B_b with γ-pyrone) and anticarcinogenic properties of saponins has shown that they may also impair the digestion of proteins, the uptake of vitamins and minerals in the gut, to act as antifungal (such as some asparagus saponins) and antiviral agents. For example, the ability of ginseng (*Panax ginseng*, Araliaceae) to slow down the aging of organism is attributed to antioxidant activity of ginsenosides, which represent a class of steroid glycosides and triterpene saponins.

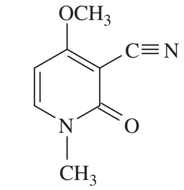
In the form of concentrates, some saponins are used as foaming agents, emulsifiers and antioxidants; glycyrrhizin from liquorice is used as a sweetener in the manufacture of confectionery and tobacco. In the manufacture of some soft drinks, as well as in the production of the famous English ginger beer, saponins from the soap tree bark (*Quillaja saponaria*, Quillajaceae) are used as foaming agents. Saponins are commonly used also in cosmetic products (shampoos and other hair preparations).

10.3.3.3 Cyanogens

Cyanogenesis is the ability of plants and some other organisms to produce hydrogen cyanide by decomposition of cyanogenic compounds. Cyanogenic compounds or cyanogens have been detected in various parts of the roughly 3000 species of plants indexed to 110 families. It is assumed that cyanogens in plants repel predators by their bitter taste and odour and the toxicity of their breakdown products. They possibly also participate in nitrogen metabolism as nitrogen storage forms during seed germination and early stages of plant development. Cyanogens are divided into three basic groups:

- cyanogenic glycosides (β-glycosides of 2-hydroxynitriles or β-glycosides of cyanohydrins)
- pseudocyanogenic glycosides (glycosides or methylazoxymethanol or azoxyglycosides)
- cyanogenic lipids (cyanolipids or fatty acid esters of cyanohydrins).

In addition to these cyanogens, some higher plants also accumulate different nitriles during the assimilation of hydrogen cyanide. Examples of these nitriles are β-cyano-L-alanine and β-aminopropionitrile in vetches (*Vicia* spp.) and sweet peas or vetchlings (*Lathyrus* spp.) of the legume family (Fabaceae) or alkaloid ricinine (**10-71**) of castor oil plant (*Ricinus communis*, Euphorbiaceae). Many plants also produce cyanides as byproducts of the biosynthesis of plant hormone ethylene (Figure 2.1).

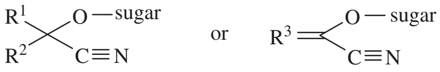


10-71, ricinine

10.3.3.3.1 Cyanogenic glycosides

Structure, nomenclature and occurrence

The approximately 75 documented cyanogenic glycosides are all *O*-β-glycosides of 2-hydroxynitriles (formerly called cyanohydrins) that are glycosides of nitriles of 2-hydroxycarboxylic acids (**10-72**). Cyanogenic glycosides are the most important and most widely occurring cyanogens of many plants consumed as human food or used as livestock feed. They are located mainly in dicotyledonous plants belonging to the families Fabaceae, Asteraceae, Euphorbiaceae and Passifloraceae and in monocotyledonous plants of the families Poaceae and Araceae. Generally, a certain cyanogenic glycoside occurs in only one or two families of plants and one or two cyanogenic glycosides occur in one plant.

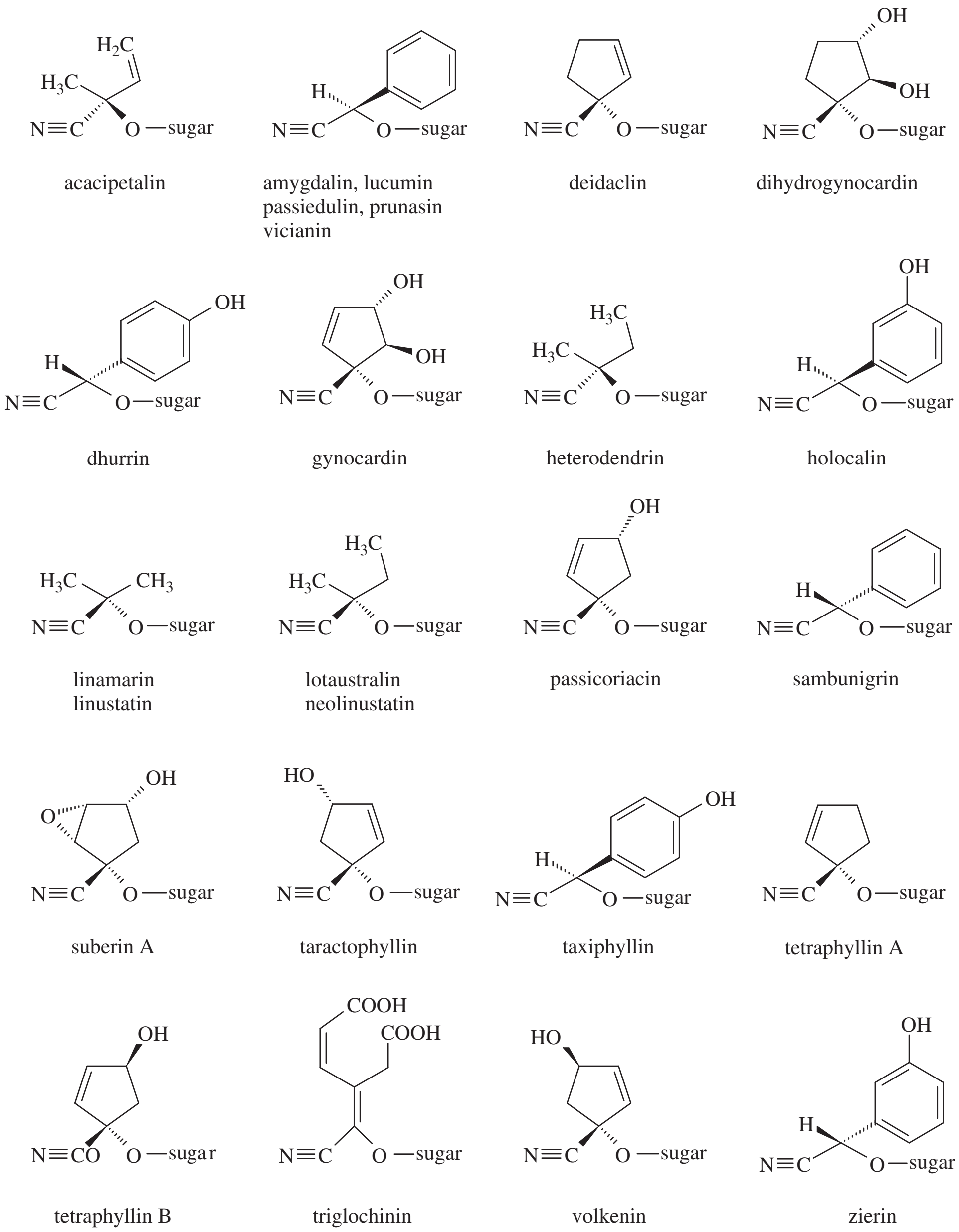


10-72, general structure of cyanogenic glycosides

Cyanogenic glycosides differ in:

- type of bound sugar
- substituents R^1, R^2 (or R^3)
- chirality of carbinol carbon atom.

With some exceptions, the sugar commonly bound in cyanogenic glycosides is monosaccharide β-D-glucose. The exceptions are disaccharides β-vicianose, α-L-Ara*p*-(1→6)-β-D-Glc*p*, β-primeverose, β-D-Xyl*p*-(1→6)-β-D-Glc*p*, β-gentiobiose, β-D-Glc*p*-(1→6)-β-D-Glc*p* and some other disaccharides. In most cyanogenic glycosides, the substituent R^1 is an aliphatic or aromatic substituent and R^2 = H. In this case the carbinol carbon atom (C-2 carbon of aglycone) is chiral and epimeric pairs of compounds therefore exist. The structure of the major cyanogenic glycosides is shown in formulae **10-73** and in Table 10.10, which also lists their occurrence.



10-73, cyanogenic glycosides

Cyanogenic glycosides are usually subdivided according to amino acids from which they arise in biosynthesis. Most cyanogenic glycosides are derived from five hydrophobic amino acids valine (linamarin), isoleucine (lotaustralin), leucine (heterodendrin and epiheterodendrin), phenylalanine (amygdalin and prunasin) and tyrosine (dhurrin, taxifyllin and triglochinin). Cyclopentanoid cyanogens (such as gynocardin) arise from cyclopentenylglycine (**2-16**).

The simplest cyanogenic glycoside derived from valine is linamarin (formerly also called phaseolunatin). Its higher homologue is (*R*)-lotaustralin, which is derived from isoleucine. Both cyanogens occur together, because the corresponding enzymes are not specific and transform both amino acids (valine and isoleucine) into cyanogenic glycosides. Of the products containing these cyanogens, the one which is the most important for human nutrition is cassava (*Manihot esculenta*, Euphorbiaceae), sometimes also called yucca or tapioca, which is a major staple food in the developing world, providing a basic diet for around 500 million people. Cassava is native to South America and is now extensively cultivated in tropical and subtropical regions, especially in sub-Saharan Africa but also in Indonesia and other countries. Cassava contains linamarin as the main cyanogenic glycoside, and (*R*)-lotaustralin is also present in an amount about 20 times smaller. Both glycosides occur in the leaves and starchy tubers, especially in the cortex. Linamarin is similarly the cyanogenic glycoside of butter (lima) beans (*Phaseolus lunatus*, Fabaceae), birds foot trefoil (*Lotus corniculatus*), white clover (*Trifolium repens*) and other plants from the same family, which are often a part of forage for livestock.

Table 10.10 Structures and occurrence of cyanogenic glycosides.

Trivial name	Sugar	Isomer	Occurrence (Latin names of plant species)
Acacipetalin (proacacipetalin)	D-Glucose	*S*	*Acacia*
Amygdalin	Gentiobiose	*R*	*Prunus*
Deidaclin	D-Glucose	*R*	*Deidamia*, *Passiflora*
Dhurrin	D-Glucose	*S*	*Sorghum*
Dihydrogynocardin	D-Glucose	1*S*,4*S*,5*R*	*Passiflora*
Gynocardin	D-Glucose	1*S*,4*S*,5*R*	*Gynocardia*, *Pangium*, *Taractogenes*, *Rawsonia*
Heterodendrin (dihydroacacipetalin)	D-Glucose	*S*	*Acacia*
Holocalin	D-Glucose	*R*	*Sambucus*
Linamarin (phaseolunatin)	D-Glucose	–	*Trifolium*, *Lotus*, *Phaseolus*, *Manihot*
Linustatin	Gentiobiose	–	*Linum*
Lotaustralin (methyllinamarin)	D-Glucose	*R*	*Lotus*, *Manihot*
Lucumin	Primeverose	*R*	*Lucuma*
Neolinustatin	Gentiobiose	*R*	*Linum*
Passicoriacin	D-Glucose	*S*	*Passiflora*
Passiedulin	D-Allose	*R*	*Passiflora*
Prunasin	D-Glucose	*R*	*Prunus*, *Malus*, *Pyrus*, *Sorbus*, *Cydonia*, *Carica*
Sambunigrin	D-Glucose	*S*	*Prunus*, *Sambucus*
Taractophyllin	D-Glucose	1*R*,4*S*	*Passiflora*
Suberin A	D-Glucose	1*R*,2*R*,3*R*,4*R*	*Passiflora*
Taxiphyllin	D-Glucose	*R*	*Taxus*, *Triglochin*, *Bambusa*
Tetraphyllin A	D-Glucose	*S*	*Tetrapathaea*, *Passiflora*
Tetraphyllin B	D-Glucose	1*S*,4*S*	*Tetrapathaea*, *Passiflora*, *Mathurina*, *Carica*
Triglochinin	D-Glucose	–	*Triglochin*, *Glyceria*, *Melica*
Vicianin	Vicianose	*R*	*Vicia*
Volkenin	D-Glucose	1*R*,4*R*	*Passiflora*, *Mathurina*
Zierin	D-Glucose	*S*	*Sambucus*

The main cyanogen in butter beans and birds foot trefoil is (*R*)-lotaustralin. Linustatin has the same aglycone as linamarin and the same aglycone as in (*R*)-lotaustralin is found in (*R*)-neolinustatin. These glycosides, containing disaccharide gentiobiose, occur as the major cyanogenic glycosides in the seeds of linseed (*Linum usitatissimum*, Linaceae). From leucine β-glucosides (*S*)-heterodendrin (dihydroacacipetalin) and (*S*)-acacipetalin (proacacipetalin) are derived, occurring in many acacias (*Acacia* spp.), shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae.

A number of cyanogenic glycosides are derived from the aromatic amino acids phenylalanine and tyrosine. From phenylalanine is derived the epimeric pair of β-glucosides known as (*R*)-prunasin and (*S*)-sambunigrin. (*R*)-Passiedulin of purple passion fruit, also known as purple granadilla (*Passiflora edulis*, Passifloraceae), originating in tropical South America, contains a rare hexose D-allose instead of D-glucose. Prunasin is a cyanogenic β-glucoside occurring in seeds of many plants of the rose family (Rosaceae), such as plums (*Prunus* spp.), apricots (*Armeniaca* spp.), peaches (*Persica* spp.), apples (*Malus* spp.), pears (*Pirus* spp.), quinces (*Cydonia* spp.) cranberries (*Sorbus* spp.), cotoneaster berries (*Cotonoaster* spp.), many acacia species and is also present in passion fruits accompanied by other cyanogens. Sambunigrin, accompanied by prunasin and other glycosides, is the main cyanogenic β-glucoside of elderberries (*Sambucus nigra*, Adoxaceae). It is found in all parts of the plant, especially in leaves and immature fruits. During maturation, its content decreases and at the time of maturity sambunigrin is not even present in the fruits. Juice made from unripe elderberries does not contain sambunigrin at concentrations that represent a health risk, but can be sensorially unacceptable for

its bitterness. (*R*)-Epiheterodendrin occurs in some varieties of malting barley (*Hordeum* spp., Poaceae) and in malt.

The same aglycone as prunasin (D-mandelic acid nitrile) contains (*R*)-amygdalin, which is β-gentiobioside, (*R*)-vicianin, containing β-vicianose, and (*R*)-lucumin, which contains β-primeverose. The most important cyanogenic glycoside of this group of cyanogens is amygdalin, since it is present in plants of the rose family (Rosaceae), together with prunasin and sambunigrin. Significant resources are bitter almonds and apricot, peach, plum and cherry pits. In small amounts, amygdalin also occurs in apple, pear and quince seeds. The precursor of amygdalin is prunasin, which is also present in the flesh of immature fruits. The amount of amygdalin is not high, and is not life threatening. Poisoning usually occurs only accidentally, for example when children consume large quantities of bitter almonds.

From phenylalanine is derived the cyanogenic β-glucoside vicianin found in vetches, such as the common vetch (*Vicia sativa*, Fabaceae). Lucumin is present in tropical plants of the genus *Lucuma* of the same plant family. Other cyanogenic β-glucosides are (*R*)-holocalin and its epimer (*S*)-zierin, which occur in small amounts in elder leaves and unripe berries.

Tyrosine yields β-glucoside (*R*)-taxiphyllin and epimeric (*S*)-dhurrin. Taxiphyllin is present in bamboo shoots (*Bambusa* spp., Poaceae), needles of yew (*Taxus baccata*, Taxaceae) and juniper (*Juniperus* spp., Cupressaceae). Dhurrin occurs in grasses (Gramineae), such as sorghum (*Sorghum bicolor*), especially in young green parts of the plants. Tyrosine is also the precursor of β-glucoside triglochinin, which occurs in some grasses, for example, in melic grasses (*Melica* spp.), reed manna grass (*Glyceria maxima*) and wetland plants, such as marsh arrow grass (*Triglochin palustris*, Junkaginaceae), which may become a part of forage for livestock.

An unusual cyanogenic glycoside is (1*S*,4*S*,5*R*)-gynocardin. It occurs along with other β-glucosides and fatty acids with cyclopentene ring (see Section 3.3.3.3.3) in the seeds of chaulmoogra trees of the genus *Hydnocarpos* (syn. *Taraktogenos*, Achariaceae, formerly Flacourtiaceae), native to Indonesia, Malaysia and the Philippines.

The content of cyanogenic glucosides in certain foods, expressed in the amount of bound hydrogen cyanide (cyanogenic potential), is given in Table 10.11. Content depends on the degree of maturity and many other factors.

Reactions and changes

Cleavage of cyanogenic glycosides may occur either enzymatically or chemically. Glycosides containing β-D-glucose are more or less hydrolysed by specific β-glucosidases that are often referred to by common names according to origin. For example, the bitter almond enzyme is called amygdalase (amygdalinase, formerly also emulsin) and the cassava enzyme is known as linamarase. The enzyme hydrolyses the glycosidic bond to produce sugar and cyanohydrin. Glycosides containing disaccharides are broken down in two stages, in the first stage the corresponding glycoside (containing bound monosaccharide) reults and in the second stage the monosaccharide and aglycone (cyanohydrin) is released. For example, prunasin arises as an intermediate from amygdalin, and is further hydrolysed to glucose and 2-hydroxynitrile. Another enzyme involved in the decomposition of cyanogenic glycosides is aldehyde lyase, which catalyses the cleavage of a cyanohydrin to aldehyde or ketone and hydrogen cyanide (Figure 10.3). Such enzymes are cyanohydrin lyase, which catalyses the cleavage of acetone cyanohydrins, mandelonitril lyase, also known as (*R*)-oxynitrilase, which cleaves a number of aromatic and aliphatic cyanohydrins,

Table 10.11 HCN content in some plant cyanogenic glycosides.

Origin	HCN (mg/kg of fresh material)	Origin	HCN (mg/kg of fresh material)
Cassava		***Stone fruits***	
Leaves	650–1 040	Bitter (sweet) almonds	2800–4110 (0–100)
Whole tubers	550	Apricot stones	3200
Tuber bark	840–2 450	Sour cherry stones	3540
Tuber flesh	100–330	Sour cherry flesh	10
Bamboo		***Sorghum***[a]	
Unripe shoots	3000	Germinating plants	2400
Tops of unripe shoots	8000	Young leaves	600
Purple passion fruit		***Legumes***	
Unripe fruit	700	Butter beans	100-4000
Ripe fruit	100	Common beans	20
Linseeds	200–380	Peas	23

[a] *Sorghum bicolor* (Poaceae).

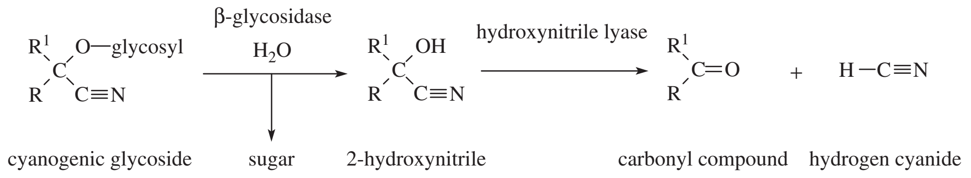


Figure 10.3 Enzymatic hydrolysis of cyanogenic glycosides.

and hydroxymandelonitril lyase that cleaves (*S*)-4-hydroxymandelo nitriles. The final product of triglochinin degradation is not the corresponding carbonyl compound, but a product of its hydration, which is (*E*)-but-2-ene-1,2,4-tricarboxylic acid.

The hydrolysis products of major cyanogenic glycosides are summarised in Table 10.12. In all cases, other products are sugars and hydrogen cyanide. The enzymatic degradation of amygdalin is shown in Figure 10.4 as an example. Hydrogen cyanide released from stone fruit cyanogens is a precursor of toxic ethyl carbamate, which occurs mainly in stone fruit distillates (see Section 12.2.8).

Cyanogenic glycosides are hydrolysed also by diluted acids at elevated temperatures, producing sugars and 2-hydroxy nitriles. For example, amygdalin hydrolysis produces D-mandelic acid nitrile. In concentrated acids, the hydrolysis of nitriles yields 2-hydroxycarboxylic acids and ammonium salts. Amygdalin thus yields D-mandelic acid. In slightly alkaline solutions, epimerisation of cyanogenic glycosides occurs. For example (*R*)-amygdalin yields a mixture of epimers known as isoamygdalin. The epimer of amygdalin derived from L-mandelic acid, called (*S*)-neoamygdalin, does not occur in nature. The epimeric pair of prunasin and sambunigrin was formerly called prulaurasin. In strongly alkaline media, both epimers yield glycosides of 2-hydroxycarboxylic acids. For example, the product of amygdalin alkaline hydrolysis is an epimeric mixture of mandelic acid gentiobioside, which is called amygdalinic acid (Figure 10.5). (*S*)-Epilucumin, which arises from (*R*)-lucumin, also does not occur in nature.

Detoxication of cassava in culinary practice is done by sun drying, crushing and grinding, soaking in water, boiling and fermentation. During fermentation, about 90% of cyanogens are removed and simultaneously formed substances carry the favourable organoleptic properties of the products. After 30 min of cooking, the content of cyanogens decreases by about 8–30% of the original amount.

Table 10.12 Hydrolysis products of cyanogenic glycosides.

Glycoside	Carbonyl compound	Glycoside	Carbonyl compound
Linamarin, linustatin	Acetone	Vicianin	Benzaldehyde
Lotaustralin, neolinustatin	Butan-2-one	Holocalin	3-Hydroxybenzaldehyde
Prunasin	Benzaldehyde	Zierin	3-Hydroxybenzaldehyde
Sambunigrin	Benzaldehyde	Taxiphyllin	4-Hydroxybenzaldehyde
Amygdalin	Benzaldehyde	Dhurrin	4-Hydroxybenzaldehyde

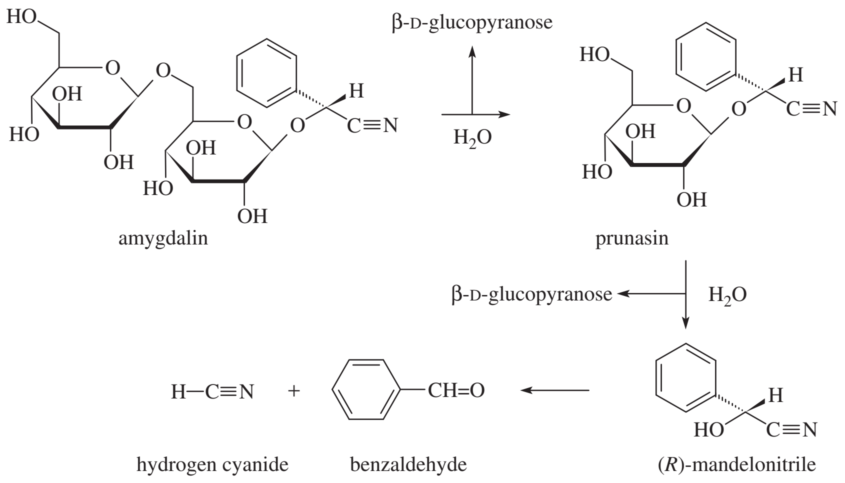


Figure 10.4 Enzymatic hydrolysis of amygdalin.

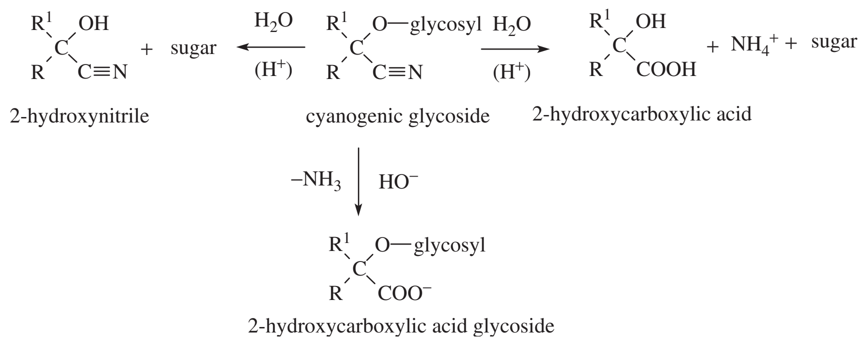


Figure 10.5 Degradation of cyanogenic glycoside in acidic and alkaline media.

Research on developing cassava with lower cyanogenic glucoside content is another way to solve the problem of cassava detoxication. The main cyanogen of bamboo shoots, taxiphyllin, is labile and decomposes during cooking.

The most common source of cyanogens in the European diet is stone fruits. Cyanogens are mainly present in the inedible part of the fruit, the stones. When whole fruit is processed, cyanogens may gradually pass from the stones into the edible part of the fruit. For example, fruit juices obtained from unstoned fruit may contain up to 15 mg/kg of hydrogen cyanide. Higher concentrations also occur in compotes made of unstoned fruit. For example, apricot compotes may contain up to 33 mg/kg of hydrogen cyanide. The amount of released hydrogen cyanide depends on the technological conditions, especially the temperature. The enzymatic degradation can occur only if there is thermal damage of cell membranes and enzymes have not been inhibited. The higher the inactivation effect, the lower the concentration of hydrogen cyanide in the finished product. Benzaldehyde arising from the enzymatic degradation of stone fruit cyanogens is often the bearer of flavour in the stone fruit products. Benzoic acid formed by partial oxidation of benzaldehyde has antimicrobial properties.

Kernels of some stone fruits (such as bitter almonds) are used to make marzipan, almond jelly and other confectionery products. Again, even in this case enzymatic hydrolysis of cyanogenic glycosides releases hydrogen cyanide, which is, however, released in the subsequent technological operations. Similar changes of cyanogens occur in the production of fruit spirits, as the major quantity of hydrogen cyanide formed in the fermentation process is vaporised during fermentation and subsequent distillation. Stone fruit distillates usually contain 0.3–3 mg/kg of hydrogen cyanide (cyanides).

Biological effects

Cyanogenic glycosides are not toxic; only their decomposition product, hydrogen cyanide, is toxic. Acute toxicity is due to inhibition of cytochrome oxidase in the respiratory chain by cyanide (reaction with copper ions) and reaction with haemoglobin (formation of cyanohaemoglobin). The lethal dose of cyanide for humans is from 0.5 to 3.5 mg/kg body weight, which corresponds to 35–245 mg for an adult weighing 70 kg. Symptoms of ingestion of a lethal dose are manifested by stiffness of limbs, dazed consciousness, cyanosis (bluish discoloration of the skin), convulsions and coma. Ingestion of lower cyanide concentrations leads to headaches, anxiety and uneasiness in the throat and chest, heartbeat and muscle weakness. Chronic symptoms of poisoning (manifested by disease of the peripheral nerves) are called degenerative tropical neuropathy.

In cases where fresh cyanogenic plants containing intact glycosides are eaten, there is only a small amount of hydrogen cyanide released, since glycosidases hydrolysing cyanogenic glycosides are inhibited in the stomachs of humans and other monogastric animals. In ruminants (polygastric animals), the plant enzymes are not inhibited and cyanogenic glycosides are partly decomposed by the rumen microflora, which can lead to poisoning.

Detoxification products of cyanides in the human body are thiocyanate (rhodanide) ions. The conversion of cyanides to thiocyanates in the presence of thiosulfates as sulfur donors is catalysed by mitochondrial rhodanese (thiosulfate : cyanide sulfurtranferase). Certain anticarcinogenic effects of cyanogenic glycosides (named vitamin B_{17}) described in the past, have not been demonstrated (see Section 5.15). It was assumed that cyanides attack cancer cells in the tissue with reduced activity of rhodanese. Another enzyme is 3-mercaptopyruvate sulfurtransferase that uses mercaptopyruvic acid as a sulfur donor. Plants have different detoxification mechanisms from animals. Cysteine (or serine) reacts with hydrogen cyanide in a reaction catalysed by β-cyanoalanine synthase with the formation of β-cyanoalanine, which is transformed by β-cyanoalanine hydratase into asparagine, an amino acid important for nitrogen storage (Figure 2.2). Asparagine can be further metabolised to aspartic acid and ammonia by the action of asparaginase. Pathogenic fungi that attack plants, such as *Gloecercospora sorghi* and *Colletotrichum graminicola* infecting sorghum, contain the enzyme cyanide hydratase catalysing the addition of water to hydrogen cyanide, which yields formamide ($H–CONH_2$).

10.3.3.3.2 Pseudocyanogenic glycosides

Toxic pseudocyanogenic glycosides (azoxyglycosides) are found in many plants of the cycad family Cycadaceae found across the subtropical and tropical parts of the world. The pseudocyanogenic glycoside cycasin occurs in seeds of some cycad species used as a source of starch. Depending on the type of plants, starchy dishes made from cycad seeds may contain up to 0.22 mg/kg of azoxyglycosides.

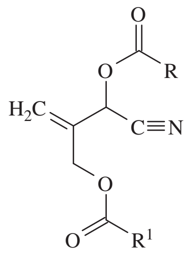
For example, sago cycad (*Cycas revoluta*), the only native cycad in Japan, has been used as a starch source on Amami Oshima, an island in the Amami Islands, for centuries. The stems are mashed into a pulp and then fermented, thus slowly removing the toxins. Likewise, seeds have been used to make a cycad cake called *sotetsu mochi*. Moreover, crushed seeds are made into *sotetsu miso*, a paste much like the normal *miso* made from soya beans. All of these practices are considered dangerous, but to this day, these traditions persist in local areas. If eaten, cycad products containing azoxyglycosides may lead to gastrointestinal distress and liver failure. Cycasin and other azoxyglycosides are not mutagenic or carcinogenic, but the main metabolite methylazoxymethanol is. Incidence of neurological disorders resembling Parkinson's disease has been reported in various areas of the Pacific (e.g. in the United States island territory of Guam), but these neurological disorders might also be caused by the presence of lathyrogenic 2,4-diaminobutyric acid (see **2-41**).

Pseudocyanogenic glycosides are decomposed by pathways other than cyanogenic glycosides. In a weakly alkaline medium they release cyanides, formates and elemental nitrogen; in an acidic medium they yield formaldehyde, methanol and elemental nitrogen. Their enzymatic degradation produces methylazoxymethanol (Figure 10.6).

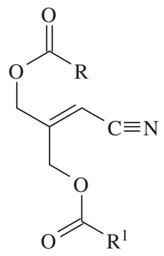
10.3.3.3.3 Cyanogenic lipids

Cyanogenic lipids (cyanolipids) occur together with conventional triacylglycerols in the seeds of many plants of the soapberry family (Sapindaceae); in certain cases they represent more than 50% of the seed oil. Members of this family have been widely studied for their pharmacological (antioxidant, anti-inflammatory and anti-diabetic properties) and anti-insect activities, and many species have economically valuable tropical fruits and wood. Some cyanogenic lipids also occur in plants of the Hippocastaneaceae and Boraginaceae families, which are not used for human consumption.

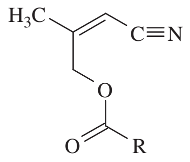
The five carbon skeletons of all cyanolipids, derived from leucine, have the cyano group and one or two hydroxyl groups esterified by fatty acids. Type I cyanolipids are diesters of 1-cyano-2-hydroxymethylprop-2-en-1-ol (**10-74**), type II cyanolipids are diesters of 1-cyano-2-hydroxy-methylprop-1-en-3-ol (**10-75**), type III cyanolipids are esters of 1-cyano-3-hydroxyprop-1-ene (**10-76**) and type IV cyanolipids are esters of 1-cyano-2-methylprop-2-en-1-ol (Figure 10.7). In seeds of some



10-74, 1-cyano-2-hydroxymethylprop-2-en-1-ol diesters



10-75, 1-cyano-2-hydroxymethylprop-1-en-3-ol diesters



10-76, 1-cyano-3-hydroxyprop-1-ene esters

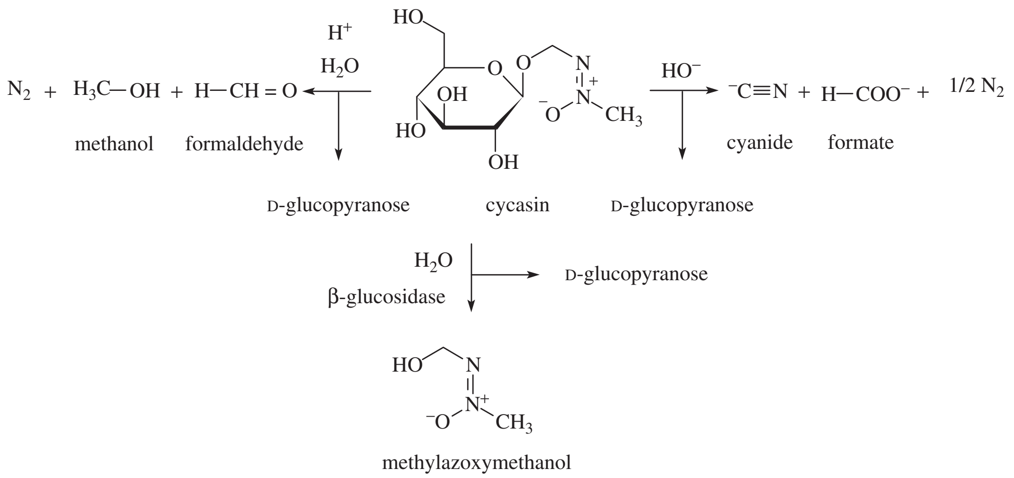
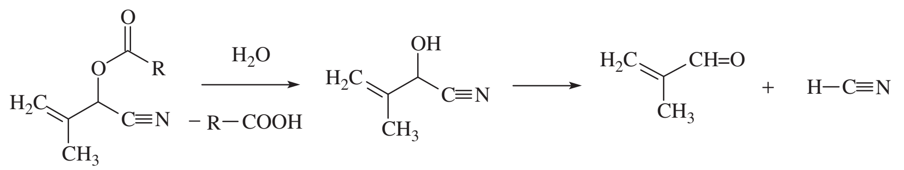


Figure 10.6 Degradation of pseudocyanogenic glycosides.



1-cyano-2-methylbut-2-en-1-ol 2-methylprop-2-enal hydrogen cyanide

1-cyano-2-methylprop-2-en-1-ol esters

Figure 10.7 Hydrolysis of cyanogenic lipids.

plants, these basic skeletons are not esterified with fatty acids, but exist as glucosides. The most common compounds are type I cyanolipids, which are often accompanied by type II cyanolipids. Compounds of type III are only found together with type II compounds. Cyanolipids of type IV are relatively rare. Compounds of types I and IV are cyanohydrins with a chiral centre, compounds of types II and III are α,β-unsaturated nitriles that are not optically active. The cyanohydrins released by acid or base hydrolysis of types I and IV cyanolipids spontaneously decompose to form hydrogen cyanide (Figure 10.7), parent carbonyl compounds and fatty acids.

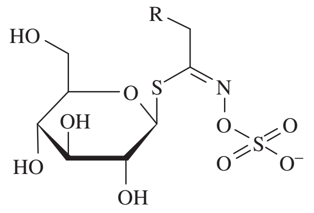
10.3.3.4 Glucosinolates

Glucosinolates, formerly called thioglucosides or mustard oil glycosides, are an important group of more than 150 secondary metabolites found exclusively in flowering dicotyledonous plants belonging to the order Brassicales. In terms of their importance, the dominant position is occupied by the crucifers (mustards or cabbage) family (Brassicaceae), which includes economically important crops (oilseeds, vegetables and condiments). For example, glucosinolates are responsible for the typical pungent taste and odour of rapeseed, mustard, horseradish and other types of vegetables and some spices.

Considerable attention was given to glucosinolates in the 1960s, mainly in connection with strumigenic (goitrogenic) effects of rapeseed meal used as feed for livestock. Glucosinolates were therefore classified as antinutritional factors that interfere with iodine metabolism. Current research is focused primarily on the biological properties of glucosinolate degradation products and their positive effects, but they also have some negative effects on human health.

10.3.3.4.1 Structure, nomenclature and occurrence

The glucosinolate molecule (**10-77**) is formed by the sugar component (in most cases β-D-glucose or β-D-thioglucose, respectively, which may be esterified by sinapic or some other carboxylic acid) and the aglycone, which is a sulfonated oxime anion in the (*E*)-position to the side chain R and in the (*Z*)-position to thioglucose residue. Generally, glucosinolates are found in nature as potassium salts. A vast number of glucosinolates occurring in nature is due to the chemical diversity of the side chain, which is derived from a few amino acids (methionine and its higher homologues, phenylalanine, tyrosine and tryptophan). An overview of main glucosinolates is given in Table 10.13.



10-77, general structures of glucosinolates

Semisystematic names of glucosinolates are formed from the chemical names of the variable part of the molecule (the side chain R) and extension glucosinolate (e.g. 2-hydroxybut-3-en-1-ylglucosinolate). However, trivial names are used more often, derived mostly from the Latin names of plants, from which glucosinolates were first isolated. For example, 4-hydroxybenzylglucosinolate was first isolated from white mustard seeds (*Leucosinapis alba*, syn. *Brassica alba*) and was therefore termed sinalbin. Prop-2-en-1-ylglucosinolate, isolated from seeds of black mustard (Brassica nigra, syn. *Sinapis nigra*), was called sinigrin. 2-Hydroxybut-3-en-1-ylglucosinolate, which is a common constituent of cruciferous vegetables and rapeseeds, is called progoitrin as it is the precursor of goitrin, showing significant antithyroid (strumigenic) effects.

The side chain R determines the chemical, physical and biological properties of the individual glucosinolates and the type of their degradation products. According to the structure of the side chain, glucosinolates can be divided into several groups:

- aliphatic or alk(en)ylglucosinolates (No. 1-6 in Table 10.13) or aliphatic hydroxy substituted glucosinolates (No. 7–9);
- sulfur-containing with a methylthio group in the side chain (No. 10–13) or their oxidised forms (No. 14–19);
- aromatic with unsubstituted or substituted benzene rings (No. 20–26);
- indole (with a substituted indole skeleton, No. 26–30).

An overview of significant plants of the Brassicaceae family that contain glucosinolates is given in Table 10.14. Some plants belonging to other families are also occasionally consumed, such as the caper bush (*Capparis spinosa*, Capparidaceae) with edible flower buds known as capers used as a seasoning, which contain

Table 10.13 Names and structure of most widespread glucosinolates.

No.	Trivial name	Substituent R	No.	Trivial name	Substituent R
1	Glucocapparin	Methyl	16	Glucoraphanin	4-Methylsulfinylbutyl
2	Sinigrin	Prop-2-en-1-yl (allyl)	17	Glucoalyssin	5-Methylsulfinylpentyl
3	Glucoputranjivin	Isopropyl	18	Glucocheirolin	3-Methylsulfonylpropyl
4	Gluconapin	But-3-en-1-yl	19	Glucoerysolin	4-Methylsulfonylbutyl
5	Glucochlearin	*Sec*-butyl	20	Glucotropaeolin	Benzyl
6	Glucobrassicanapin	Pent-4-en-1-yl	21	Sinalbin	4-Hydroxybenzyl
7	Progoitrin	(*R*)-2-hydroxybut-3-en-1-yl	22	Glucolimnantin	3-Methoxybenzyl
8	Epiprogoitrin	(*S*)-2-hydroxybut-3-en-1-yl	23	Glucoaubrietin	4-Methoxybenzyl
9	Gluconapoleiferin	2-Hydroxypent-4-en-1-yl	24	Gluconasturtiin	2-Phenethyl
10	Glucoibervirin	3-Methylthiopropyl	25	Glucobarbarin	(*R*)-2-Hydroxy-2-phenethyl
11	Glucoraphasatin	4-Methylthiobut-3-en-1-yl	26	Glucosibarin (epiglucobarbarin)	(*S*)-2-Hydroxy-2-phenethyl
12	Glucoerucin	4-Methylthiobutyl	27	Glucobrassicin	3-Indolylmethyl
13	Glucoberteroin	5-Methylthiopentyl	28	4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl
14	Glucoiberin	3-Methylsulfinylpropyl	29	Neoglucobrassicin	1-Methoxy-3-indolylmethyl
15	Glucoraphenin	4-Methylsulfinylbut-3-en-1-yl	30	4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl

Table 10.14 Overview of dominant glucosinolates of selected vegetables and oilseeds.

Vegetable	Latin name	Typical glucosinolates (names of substances are given in Table 10.13)
Cabbage	*Brassica oleracea* convar. *capitata*	2, 14, 26, 28
Brussels sprouts	*Brassica oleracea* convar. *oleracea* var. *gemmifera*	2, 14, 26, 28, 29
Cauliflower	*Brassica oleracea* var. *botrytis*	2, 4, 7, 18, 26, 27, 29
Cale	*Brassica oleracea* var. *sabellica*	2, 7, 26
Kohlrabi	*Brassica oleracea* var. *gongylodes*	2, 14, 26, 28
Broccoli	*Brassica oleracea* var. *italica*	4, 7, 10, 12, 14, 16, 17, 27, 28, 29, 30
Chinese cabbage	*Brassica chinensis* var. *chinensis*	23, 26
Turnip rape	*Brassica rapa* subsp. *rapa*	2, 4, 7, 24, 26, 27
Rapeseed[a]	*Brassica napus* var. *napus*	4, 6, 7, 9, 27
Brown mustard	*Brassica juncea*	2, 4
Black mustard	*Brassica nigra*	2
White mustard	*Leucosinapis alba*	21
Radishes	*Raphanus sativus*	11, 15, 16, 24, 27, 28
Horseradish	*Armoracia rusticana*	2, 21
Garden cress	*Lepidium sativum*	20

[a]Crambe (*Crambe abyssinica*), an oilseed crop native to the Mediterranean area, contains glucosinolate epiprogoitrin as the main instead of progoitrin. Rapeseeds contain a number of other minor glucosinolates. For example, the 00 varieties (varieties with low erucic acid and glucosinolate contents) contain, in addition to the above compounds, glucosinolates 1, 8, 14–18, 21, 23–26, 28, 29 (Table 10.13), 2-hydroxy-2-methylpropyl glucosinolate, 2-hydroxybenzyl glucosinolate and glycosides of glucosinolates with sugar bound to the hydroxyl group of the aromatic ring (2-α-L-rhamnopyranoside of 2-hydroxybenzyl glukosinolate), glucosinolate esters with phenolic acids bound to glucose, for example, 6′-sinapoylglucoraphenin and (*Z*)-6′-feruloylglucosibarin.

glucosinolate glucocapparin. The seeds of papaya (*Carica papaya*, Caricaceae), containing glucosinolate glucotropaeolin, are used for their spicy and pungent flavour as a black pepper substitute.

The intake of glucosinolates by farm animals is associated with non-traditional *Brassica* fodder crops (such as fodder kale, fodder rape, swede and turnip) and rapeseed meal, a byproduct from crushing, expelling and extracting oil from oilseed rape. In human food, the income of glucosinolates is almost exclusively associated with the consumption of cruciferous vegetables. The most important source of glucosinolates are cabbage, cauliflower, kale, broccoli and Brussels sprouts, followed by occasionally consumed vegetables, such as radishes, watercress, horseradish and others. Vegetables of the cruciferous family commonly contain approximately 20–30 glucosinolates. In one plant there are usually only a few of them in significant quantities (Table 10.14). While the composition of glucosinolates for each plant (or variety) is typical to a certain extent and is determined mainly by genetic factors, the total glucosinolate content is influenced by a number of external factors during cultivation (such as climate conditions, use of fertilisers, pest attack and other factors). The average glucosinolate content in the vegetative parts of plants (in fresh vegetables) ranges from 100 to 2500 mg/kg, but in seeds may reach values of up to 60 000 mg/kg.

10.3.3.4.2 Reactions and changes

In plant tissue, glucosinolates are accompanied by the enzyme myrosinase (thioglucoside glucohydrolase), which catalyses their decomposition. Myrosinase is a globular glycoprotein with a relative molecular weight of 140 kDa, consisting of several isoenzymes. A special feature is the occurrence of myrosinase in some microorganisms (fungi) and even in some animal tissues. In intact tissue, myrosinase is located separately from glucosinolates, but in mechanically damaged cells (e.g. cells which have been damaged by cutting, biting or freezing) it comes into contact with glucosinolates, which are then hydrolysed relatively rapidly. The rate of glucosinolate hydrolysis is determined by enzyme activity, which is influenced by many factors (temperature, pH value, type and part of the plant or the presence of substances which act as activators or inhibitors).

The actual enzymatic hydrolysis is initiated by myrosinase-catalysed cleavage of the thioglucoside bond of glucosinolate. Hydrolysis yields, in addition to D-glucose, an unstable intermediate (aglycone) thiohydroxamate-*O*-sulfonate, which is spontaneously degraded with the cleavage of bisulfate ion (HSO_4^-) and stabilisation of the remaining part of the molecule yielding one or more stable products (Figure 10.8). The most common glucosinolate decomposition products are isothiocyanates and nitriles: however, depending on glucosinolate structure and some external factors, a number of other products can arise. Isothiocyanates (and partly also nitriles) formed by hydrolysis of aliphatic glucosinolates contribute to the typical spicy flavour of cruciferous vegetables (see Section 8.3.7.5).

For example, degradation of the most common glucosinolate, sinigrin, yields, in addition to allyl isothiocyanate (see Section 8.2.9.1.5) and allyl cyanide (but-3-enenitrile, see Section 8.2.7.1.3), allyl thiocyanate (it is not clear whether this substance arises directly from the unstable aglycone or by isomerisation of isothiocyanate) and 2-(cyanomethyl)thiirane, also known as

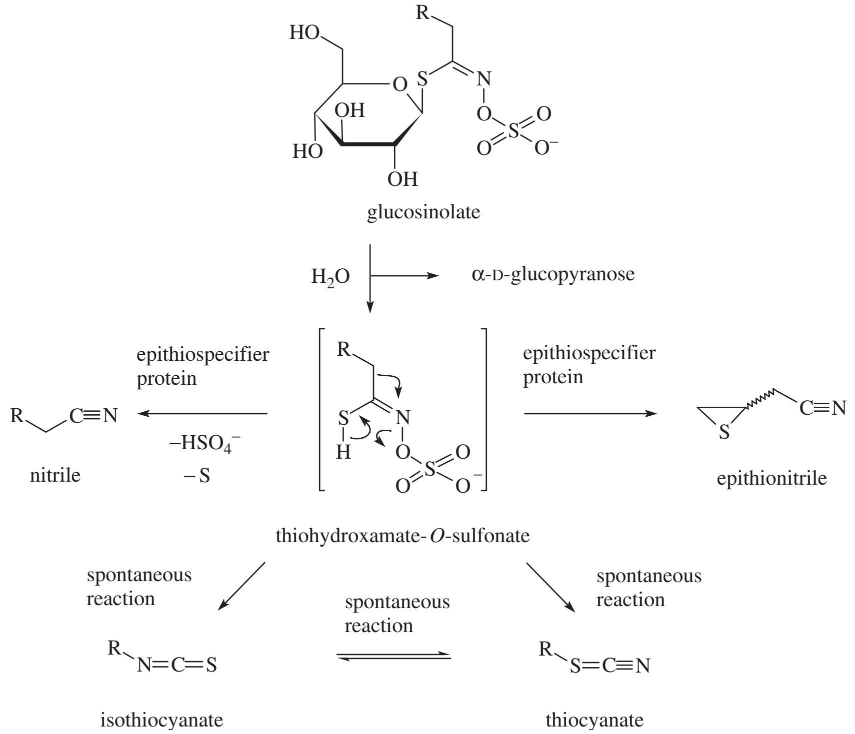
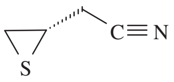


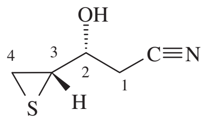
Figure 10.8 General mechanism of glucosinolate degradation.

2-(cyanomethyl)episulfide or 1-cyano-2,3-epithiopropane (**10-78** and **10-79**), which arises at the expense of allylcyanide in the presence of a special protein cofactor, called epithiospecifier protein (ESP), whose existence has been demonstrated in many plants. This protein is essential for myrosinase induced formation of (*R*)- and (*S*)-enantiomeric epithionitriles of all glucosinolates with unsaturated side chains. For example, gluconapin yields both enantiomers of 2-(thiiran-2-yl)acetonitrile (1-cyano-3,4-epithiobutane), from glucobrassicanapin arises 1-cyano-4,5-epithiopentane, from progoitrin, except (*R*)-1-cyano-2-hydroxybut-3-ene, arise diastereoisomeric (*R*)-1-cyano-2-hydroxy-3,4-epithiobutanes (3-hydroxy-4,5-epithiopentylnitriles, **10-80** and **10-81**) and from gluconapoleiferin 1-cyano-2-hydroxy-4,5-epithiopentanes. Isothiocyanates resulting from 2-hydroxyalkenylglucosinolates are unstable and spontaneously cyclise (by intramolecular addition of hydroxyl group) to substituted 5-alkenyl-1,3-oxazolidine-2-thiones. Progoitrin, the dominant glucosinolate of rapeseeds, yields toxicologically significant (*R*)-5-vinyl-1,3-oxazolidine-2-thione called goitrin (Figure 10.9), napoleiferin produces 5-allyl-1,3-oxazolidine-2-thione and glucobarbarin gives rise to 5-phenyl-1,3-oxazolidine-2-thione.

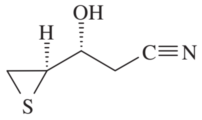
10-78, (*R*)-2-(cyanomethyl)thiirane



10-79, (*S*)-2-(cyanomethyl)thiirane

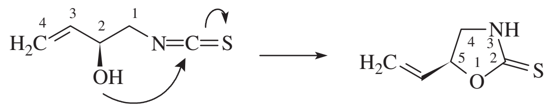


10-80, (2*R*,3*R*)-1-cyano-2-hydroxy-3,4-epithiobutane



10-81, (2*R*,3*S*)-1-cyano-2-hydroxy-3,4-epithiobutane

The situation is somewhat more complicated in the case of the so-called indole glucosinolates (indol-3-ylmethylglucosinolates) called glucobrassicins. In seeds of oilseed rape and other *Brassica* species, the total pool of glucosinolates is most often dominated by 4-hydroxyindol-3-ylmethyl glucosinolate (4-hydroxyglucobrassicin) and methionine-derived glucosinolates. In contrast, vegetative parts of *Brassica* species, including various types of vegetables, have a relatively high content of the indol-3-ylmethyl glucosinolates: glucobrassicin, 4-methoxyglucobrassicin

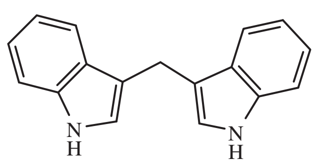


(*R*)-2-hydroxybut-3-en-1-ylisothiocyanate (*R*)-5-vinyloxazolidine-2-thione

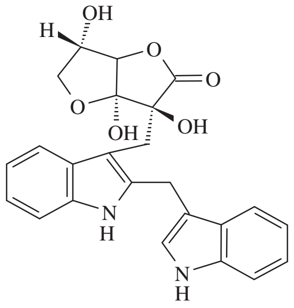
Figure 10.9 Formation of goitrin from 2-hydroxybut-2-en-1-yl isothiocyanate.

and 1-methoxyglucobrassicin (neoglucobrassicin). Under the catalysis of myrosinase, the indol-3-ylmethylglucosinolates (as well as some other arylmethylglucosinolates) are degraded to indol-3-ylacetonitriles and unstable isothiocyanates, with release of the thiocyanate (rhodanide) ion and a reactive carbonium ion, which gives a complex mixture of reaction products, such as 3-hydroxymethylindoles (indol-3-ylmethanols) and ascorbigens, depending on the nucleophiles available and the reaction conditions (Figure 10.10). Their subsequent reactions give various dimers and oligomers.

Ascorbigen derived from glucobrassicin was formerly called ascorbigen A. In vegetables rich in ascorbic acid, ascorbigen is the main transformation product of glucobrassicin. Its contents in cruciferous vegetables can reach 5–60 mg/kg. Depending on various factors, about 20–50% of glucobrassicin can be converted into ascorbigen, which has 15–20% of ascorbic acid activity. Stabilities of 3-hydroxymethylindole and ascorbigen are limited, particularly at higher temperatures, and both compounds provide a wide range of transformation products. Self-condensation of 3-hydroxymethylindole, after cleavage of formaldehyde, yields 3,3′-diindolylmethane (**10-82**) and other di- and polyindolylmethanes. At higher temperatures, ascorbigen splits off ascorbic acid and the remaining residue can bind as a cation to another molecule of ascorbigen to form a dimer (**10-83**) and a trimer (**10-84**). Binding to other nucleophilic compounds is also possible. A mixture of the dimer and trimer was previously called ascorbigen B.



10-82, 3,3′-diindolylmethane



10-83, ascorbigen dimer

Thermal degradation of glucobrassicin produces 3-indolylacetonitrile as the main product (Figure 10.10), 3-indolylacetic acid, 3-indolylacetamide, 3-formylindole (**10-85**), 3-methylindole (skatole, see Section 2.5.1.1.3) and other compounds.

In relation to the biological effects of glucosinolates, particular attention is paid to the influence of external conditions (particularly

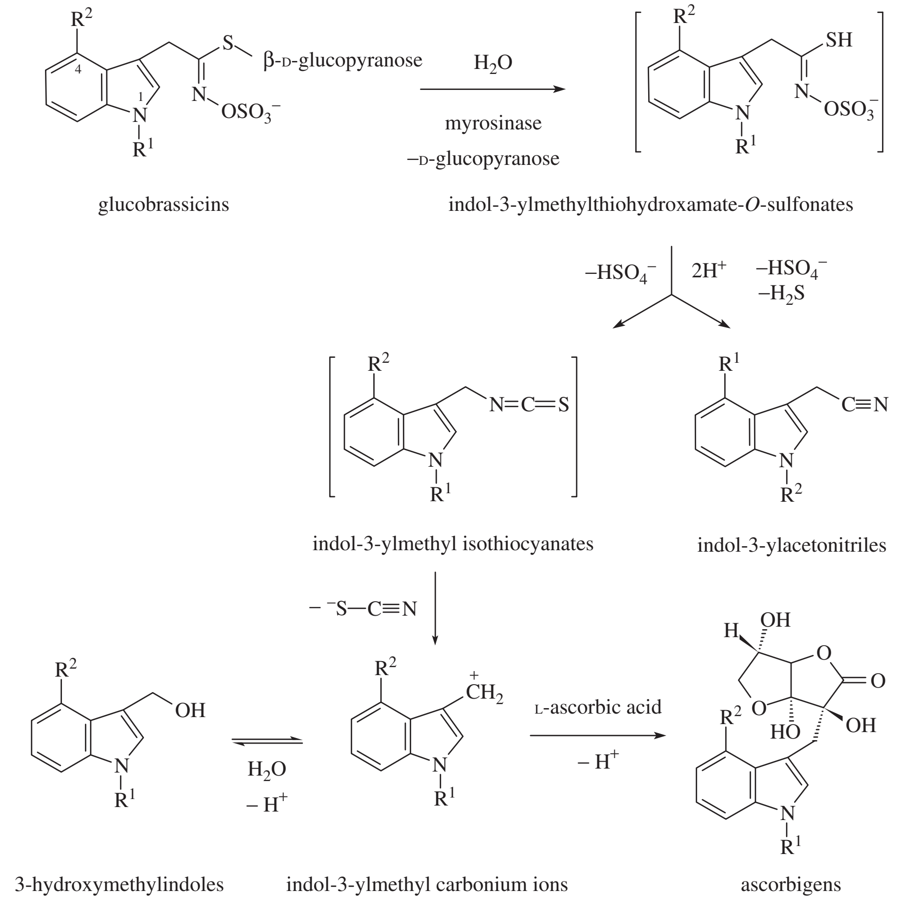
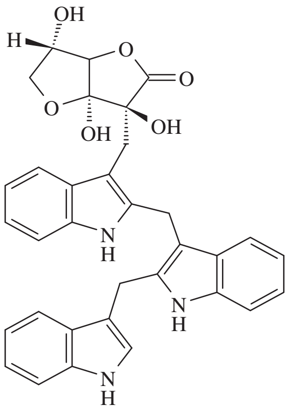
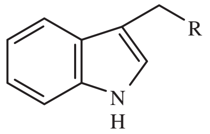


Figure 10.10 Degradation of indol-3-ylmethylglucosinolates:
Glucobrassicins: glucobrassicin ($R^1 = R^2 = H$), 4-hydroxyglucobrassicin ($R^1 = H$, $R^2 = OH$), 4-methoxyglucobrassicin ($R^1 = H$, $R^2 = OCH_3$), neoglucobrassicin ($R^1 = OCH_3$, $R^2 = H$)
Ascorbigens: ascorbigen ($R^1 = R^2 = H$), 4-hydroxyascorbigen ($R^1 = H$, $R^2 = OH$), 4-methoxyascorbigen ($R^1 = H$, $R^2 = OCH_3$), neoascorbigen ($R^1 = OCH_3$, $R^2 = H$).



10-84, ascorbigen trimer



10-85, 3-indolylacetic acid, R = COOH
3-indolylacetamide, R = $CONH_2$
3-indolylacetaldehyde, R = CH=O

pH, temperature and concentration of metal ions) to the mechanism of their hydrolysis. These effects should be taken into account when assessing the potential toxicity and the positive effects of glucosinolates, because they are significantly involved in the formation of physiologically active products. The pH value of the reaction medium has an effect on the rate of enzymatic hydrolysis as myrosinases of various plants usually have several pH-optima, which lie in the interval of between pH 5 and 8, due to the presence of several isoenzymes and the degradation mechanism of unstable aglycone (Figure 10.8). Generally, under acidic conditions (pH < 4) nitriles predominantly arise, while at higher pH values larger amounts of isothiocyanates are formed. Another factor affecting the decomposition of glucosinolates is temperature. Thermal processing of vegetables (such as cooking, blanching and sterilisation) leads

to inactivation of enzymatic systems, including myrosinase and epithiospecifier protein. Depending on the temperature and duration of heat treatment, glucosinolates are also partially decomposed (the main cause of losses is leakage into water) along with some other thermolabile products (such as 3-hydroxymethylindole and ascorbigen). In addition, a number of volatile substances are evaporated, in particular low molecular weight isothiocyanates, which significantly changes the flavour characteristics of processed vegetables. The disruption of plant tissue, which was preceded by a sufficiently effective thermal intervention, no longer results in enzymatic degradation of glucosinolates and the remaining amount of glucosinolates stays in the food. Glucosinolate hydrolysis may be affected by the presence of various chemicals, of which the most significant is ascorbic acid, which occurs in cruciferous vegetables in amounts of about 100–1200 mg/kg. In addition to the formation of ascorbigen, ascorbic acid also participates in the myrosinase activation, thus speeding up the whole process of enzymatic degradation of glucosinolates. Changes in the content of glucosinolates in selected cruciferous vegetables during cooking are summarised in Table 10.15.

Generally, blanching and cooking of vegetables results in a considerable leakage of glucosinolates into water, on average, 30–40% of glucosinolates are extracted, and in cabbage 28% during 10 min of boiling. Losses of glucosinolates by leakage and degradation are lowest for sinigrin (about 30%) and highest for neoglucobrassicin (about 60%). Approximately one-third to half of the original content of glucosinolates remains unchanged during cooking. In frozen vegetables, as a result of myrosinase activity in damaged tissues, about 50% of the originally present glucosinolates decomposes. Virtually complete degradation of glucosinolates occurs during the first week of cabbage fermentation.

10.3.3.4.3 Biological effects

The mean daily intake of glucosinolates is difficult to evaluate as there are large variations between countries, income groups (e.g. it is likely that certain individuals, such as vegetarians, will consume more than 300 mg total glucosinolates per day) and over the year (the amount consumed is approximately doubled in the winter months). The nutritional and toxicological consequences of such an intake are largely unknown at present. For example, the mean total glucosinolate intake in Germany (in 2009) was 14.2 mg/day for men and 14.8 mg/day for women, and increased with age and education. Quantitatively, the most important individual glucosinolates were glucobrassicin and sinigrin, with mean daily intakes of 3.5 and 1.7 mg/day for men and 4.2 and 2.5 mg/day for women, respectively. Broccoli, Brussels sprouts and cauliflower contributed most to the total glucosinolate intake.

Glucosinolates as such are in practice indifferent compounds and thus are probably neither harmful nor beneficial. Only products of their degradation show biological effects. Degradation of glucosinolates leads to a wide variety of substances. The situation is aggravated by the fact that one substance often exhibits several biological effects. When evaluating the consequences of their presence in a particular crop, attention should therefore be paid to:

- which glucosinolates are present and in what quantities
- which products will arise by their degradation and under what conditions
- biological activity of resulting products.

Table 10.15 Glucosinolates content in fresh and cooked cruciferous vegetables.

Vegetables	Content (mg/kg fresh weight)		Content of individual glucosinolates (mg/kg fresh weight) (compound numbers are given in Table 10.13)										
	Range	Average	2	4	6	7	9	12	13	14	24	26	28
Cabbage													
Raw	360–2754	1089	263	18	-	38	-	-	-	450	-	295	25
Cooked	315–1651	786	202	13	-	27	-	-	-	300		174	11
Cauliflower													
Raw	138–2083	620	142	7	-	23	-	-	-	173	-	227	48
Cooked	94–1111	420	100	3	-	14	-	-	-	122	-	151	30
Brussel sprouts													
Raw	1455–3939	2260	445	252	-	478	-	-	-	353	-	624	110
Cooked	597–2452	1237	264	148	-	299	-	-	-	195	-	298	33
Turnip													
Raw	392–1657	560	-	42	37	371	42	46	71	-	97	48	96
Cooked	205–944	291	-	24	26	206	23	23	39	-	58	23	38

Toxic effects

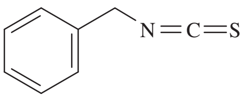
Decomposition of aliphatic glucosinolate leads to slightly strumigenic isothiocyanates, some of which exhibit strong antimicrobial and insecticidal effects (such as allyl isothiocyanate formed from sinigrin). Other products include nitriles and cyanoepithioalkanes that may be hepatotoxic and nephrotoxic. Strumigenic (*R*)-5-vinyl-1,3-oxazolidine-2-thione (goitrin), formed by progoitrin hydrolysis, inhibits the synthesis of thyroid hormones (thyroxine and triiodothyronine) and transmission of iodine in the thyroid gland. Thiocyanate anions, arising from unstable indole and aromatic isothiocyanates, exhibit moderate strumigenic activities, but unlike goitrin and isothiocyanates, they act competitively with respect to iodine. Both mechanisms can lead to an enlarged thyroid gland (goitre) and disturbances of its function. In humans, the strumigenous effects of these compounds have not been demonstrated.

The presence of glucosinolates may be a problem in fodder production. Major deleterious effects of glucosinolates ingestion in animals are reduced palatability, decreased growth and reproduction. Progoitrin and epiprogoitrin impair palatability at a level of between 2.3 and 4.7 mol/g diet, while at higher levels feed intake decreases. Nitriles are known to affect liver and kidney functions. The thiocyanates interfere with iodine availability, whereas goitrin is responsible for the morphological and physiological changes of thyroid. Rapeseed meal feeding (containing 36–40% of protein) does not impair the carcass quality, but increases erucic and elaidic acids contents in carcass and milk fat. However, the newly introduced rapeseed cultivars of canola quality (commonly known as double-low or 00) have, in addition to the low content of erucic acid (0.2%, which corresponds to about 0.5% in the oil), also reduced glucosinolate content (20 mmol/kg). Ruminants are less sensitive to dietary glucosinolates. Pigs are more severely affected by dietary glucosinolate compared with rabbits, poultry and fish. The tolerance level of glucosinolates in ruminants, pig, rabbits, poultry and fish is 1.5–4.2, 0.78, 7.0, 5.4 and 3.6 mol/g of diet, respectively. Results using techniques based on detoxification, such as removal of glucosinolates and their breakdown products by alkali extraction, exposure to water vapour at higher temperatures (toasting) are not satisfactory, especially with regard to their economic demands. Iodine supplementation in the diet of pigs and ruminants seems to be promising.

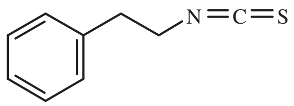
Beneficial effects

Experimental studies with laboratory animals and the results of epidemiological studies have confirmed that increased consumption of cruciferous vegetables reduces the risk of chemically induced cancer, which is cancer induced by intake of carcinogens. The active principles are some glucosinolate degradation products, although the presence of ascorbic acid, α-tocopherol, β-carotene, dimethylthiosulfinate, phenolic antioxidants (e.g. flavonoids) and fibre cannot be ignored.

Benzyl isothiocyanate (**10-86**) and 2-(phenylethyl) isothiocyanate (**10-87**), which arise by hydrolysis of gluconasturtiin and glucotropaeolin, respectively, have the ability to activate some important enzymatic system deactivating carcinogens.

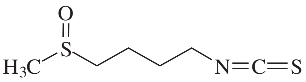


10-86, benzyl isothiocyanate



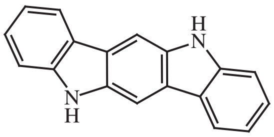
10-87, 2-(phenylethyl) isothiocyanate

Also worth mentioning is sulforaphane, 4-(methylsulfinyl)butyl isothiocyanate, also known as 4-(methanesulfinyl)butyl isothiocyanate (**10-88**), whose beneficial effects have been appreciated only recently. Its precursor is glucosinolate glucoraphanin occurring, for example, in broccoli or radishes. Another product of glucoraphanin degradation is the corresponding nitrile, which is called sulforaphane nitrile. In broccoli, glucoraphanin is the main glucosinolate. It is present at a level of about 1600 mg/kg fresh weight (approximately 55% of the total glucosinolate content). The other broccoli glucosinolates (in descending order of quantity) are glucoiberin, glucobrassicin, glucoerucin, progoitrin, 4-hydroxyglucobrassicin, glucoibervirin, 4-methoxyglucobrassicin, gluconapin, glucoalyssin and 1-methoxyglucobrassisin. The main glucosinolate of radishes is glucoraphasatin (178–2230 mg/kg fresh weight), which is followed by glucobrassicin, glucoraphenin and glucoraphanin (0–24 mg/kg fresh weight, 0–7% of the total glucosinolate content).

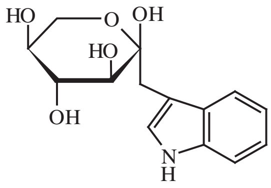


10-88, 4-(methylsulfinyl)butyl isothiocyanate

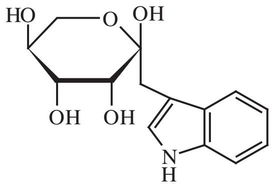
Much attention has recently been devoted to degradation products of indole glucosinolates, as they are found in virtually all commonly consumed vegetables, and sometimes form a significant proportion of glucosinolates present (e.g. they account for 95% of glucosinolate content in Chinese cabbage). One of the major degradation products of glucobrassicin is 3-hydroxymethylindole, a compound possessing an unusual ability to induce enzymes of the first and second phases of detoxification. Ascorbigen, resulting from the reaction of 3-hydroxymethylindole with ascorbic acid, is partially degraded in the stomach, ascorbic acid splits off and the molecule residue is transformed into products with antimutagenic and anticarcinogenic properties, such as 5,11-dihydroindolo[3,2-*b*]carbazole (**10-89**), which is able to inhibit certain DNA-damaging carcinogens. In neutral media, ascorbigen is converted into indole sugars (**10-90** and **10-91**), the toxicological evaluations of which, as well as the evaluation of the effects of other ascorbigen metabolites and 3-hydroxymethylindole, are not yet sufficiently developed.



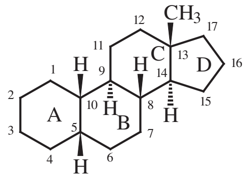
10-89, 5,11-dihydroindolo[3,2-*b*]carbazole



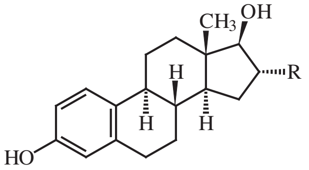
10-90, 1-deoxy-1-(3-indolyl)-α-L-sorbopyranose



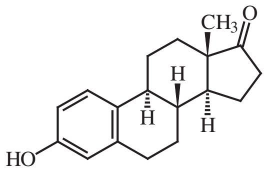
10-91, 1-deoxy-1-(3-indolyl)-α-L-tagatopyranose



10-92, 5β-oestrane



10-93, oestradiol, R = H
oestriol, R = OH



10-94, oestrone

10.3.3.5 Phenolic compounds

Phenolic compounds found in foods exhibit a wide range of biological effects. Their antimicrobial and antioxidant properties are particularly appreciated, but some phenolic compounds also show toxic effects. Some coumarins have carcinogenic effects related to their phototoxicity; oestrogenic properties are shown by some lignans, isoflavonoids (isoflavones and related pterocarpans) and prenylflavonoids, which also display anticarcinogenic effects. Other phenolic compounds such as phenolic acids (see Section 8.2.6.1.6), lignans, tannins (see Section 8.3.5), stilbenes (see Section 10.3.3.6.3), flavonoids (see Section 9.4), and many other phenolic compounds, exhibit a wide range of other biological effects.

10.3.3.5.1 Oestrogenic substances

Sex hormones are steroid compounds regulating normal development and function of reproductive human organs and organs of other animals. Male hormones are androgens, but very little is known about the plant **androgens** (testosterone and androsterone) found, for example, in the pollen of pines (*Pinus silvestris*, Pinaceae). Androgenic activity is also seen in the sweetener stevioside (see Section 11.3.2.1.3). Women (female) sex hormones are called **gynecogens** that include:

- **oestrogens** produced by the ovarian follicles
- **gestagens**, hormones with progestational activity, produced by *corpus luteum*, a temporary endocrine structure.

The most active women's oestrogen derived from hydrocarbon 5β-oestrane (**10-92**) is 3,17β-oestradiol (abbreviated E_2, **10-93**), which is supplied to the tissues by blood circulation, where it binds to specific receptors (proteins). In the body, it is transformed into oestrone (**10-94**) and oestriol (**10-93**).

Structure, nomenclature and occurrence

Oestrogenic activity is not limited to mammalian steroid hormones. In addition to endogenous oestrogens (hormones), a number of exogenous compounds present in foods of plant origin as natural components or substances that get into the food as contaminants (mould metabolites, pesticides and some other substances), also possess oestrogenic activity. These substances resemble the effect of sex hormones and induce oestrus as seen in experimental animals.

According to the origin, exogenous oestrogenic compounds include:

- **phytooestrogens**
- **mycooestrogens**
- **xenooestrogens** also known as anthropogenic oestrogens.

Phytooestrogens

Substances with oestrogenic effects (several dozens of substances are known) occur in about 300 plant species. The main phytooestrogens belong to the following groups of phytochemicals:

- isoflavones
- prenylflavonoids
- pterocarpans
- lignans.

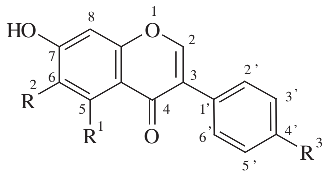
The first phytooestrogen identified in plants was oestrone, found in small quantities in palm and palm kernel oils (obtained from oil palm, *Elaeis guineensis*, Arecaceae) and pomegranate seeds (*Punica granatum*, Punicaceae).

Isoflavones

Isoflavonoids are a group of flavonoid compounds exhibiting different biological effects. The occurrence of about 200 known

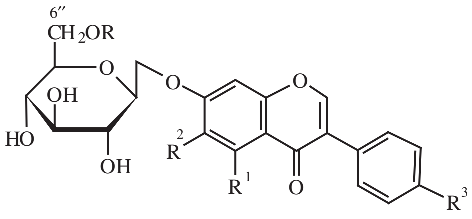
isoflavonoids is essentially limited to legumes, to which, for example, soya (*Glycine max*, Fabaceae) belongs. Isoflavones are also found in smaller quantities in some other plant families, such as Amaranthaceae, Iridaceae, Moraceae and Rosaceae. The main representatives of isoflavonoid substances exhibiting oestrogenic effects are isoflavones, compounds isomeric with flavones. Some isoflavones also exhibit antimicrobial and other biological effects.

Soya beans contain isoflavone daidzein (7,4′-dihydroxyisoflavone), which is the most active oestrogenic isoflavone, accompanied by genistein (5,7,4′-trihydroxyisoflavone), formononetin (7-hydroxy-4′-methoxyisoflavone), glycitein (7,4′-dihydroxy-6-methoxyisoflavone) and biochanin A (5,7-dihydroxy-4′-methoxyisoflavone, **10-95**). The biochemical precursor of daidzein, formononetin and glycitein is flavanone liquiritigenin (7,4′-dihydroxyflavanone), the precursor of genistein and biochanin A is naringenin (5,7,4′-trihydroxyflavanone).



10-95, isoflavones
daidzein, $R^1 = H$, $R^2 = H$, $R^3 = OH$
genistein, $R^1 = OH$, $R^2 = H$, $R^3 = OH$
formononetin, $R^1 = H$, $R^2 = H$, $R^3 = OCH_3$
glycitein, $R^1 = H$, $R^2 = OCH_3$, $R^3 = OH$
biochanin A, $R^1 = OH$, $R^2 = H$, $R^3 = OCH_3$

Isoflavones occur predominantly as 7-β-D-glucosides. The glucoside of daidzein is daidzin, the glucoside of genistein is genistin, that of formononetin is ononin, and the glucoside of glycitein is glycitin (**10-96**). The main components of soya beans are glycosides genistin, daidzin and glycitin and their malonic acid esters. In addition to these compounds, free isoflavones and acetyl derivatives of glycosides occur in small amounts, which are products of decarboxylation of malonyl esters (**10-96**). Sprouting beans contain formononetin as one of the major isoflavones. Contents of the individual compounds are given in Table 10.16. The isoflavones concentrations in soya beans vary over a wide range, from about 0.13 to 0.42%, their content in soya bean flour is about 0.2%, in soya isolates 0.06–0.10%, and 0.07% in soya concentrates. Free aglycones even occur in acid hydrolysate of soybean meal, which is used as a food seasoning.



10-96, glucosides of isoflavones and their esters
daidzin, $R = H$, $R^1 = H$, $R^2 = H$, $R^3 = OH$
genistin, $R = H$, $R^1 = OH$, $R^2 = H$, $R^3 = OH$
ononin, $R = H$, $R^1 = H$, $R^2 = H$, $R^3 = OCH_3$
glycitin, $R = H$, $R^1 = H$, $R^2 = OCH_3$, $R^3 = OH$
6″-acetylgenistin, $R = COCH_3$, $R^1 = OH$, $R^2 = H$, $R^3 = OH$
6″-malonylgenistin, $R = COCH_2COOH$, $R^1 = OH$, $R^2 = H$, $R^3 = OH$

In addition to soya beans, oestrogenic isoflavones also occur in other plants, but in much smaller quantities. For example, peanuts contain about 0.50 mg/kg of daidzein and 0.83 mg/kg of genistein (free and bound as glycosides and their derivatives). Sunflower seeds contain 0.08 mg/kg of daidzein and 0.14 mg/kg of genistein, poppy seeds contain 0.18 and 0.07 mg/kg of daidzein and genistein, respectively. Formononetin is also the main isoflavone of some forage plants. For example, red clover (*Trifolium pratense*) of the legume family (Fabaceae) contains 1–30 g/kg of formononetin in dry matter. Minority forage isoflavones are also genistein, daidzein and biochanin A. Daidzein-8-*C*-glucoside called puerarin is found in a number of plants and herbs such as the root of kudzu plant

Table 10.16 Isoflavones and their derivatives content in soya beans and soya bean products (mg/kg).

	Glucoside			Aglykon			Malonate			Acetate		
Product[a]	Daidzin	Genistin	Glycitin	Daidzein	Genistein	Gycitein	Daidzin	Genistin	Glycitin	Daidzin	Genistin	Glycitin
Beans	234–637	326–888	60–66	10–28	11–30	19–22	121–690	290–1 756	58–72	trace	2–5	25–33
Flour	147	407	41	4	22	19	261	1023	57	trace	1	32
Isolate	trace–88	137–301	34–49	11–63	36–136	25–53	18–20	88–100	36–39	6–74	0–215	33–46
Concentrate	Tr	18	31	0	0	23	0	Tr	0	Tr	1	0
Tofu[b]	25	84	8	46	52	12	159	108	0	8	1	29
Tempeh[c]	2	65	14	137	193	24	255	164	0	11	0	0
Miso[d]	0–72	96–123	18–21	34–271	93–183	15–54	0	0	19–22	1	2–11	0

[a]Tr = traces.
[b]Tofu (also known as bean curd), originating in ancient China, is made by coagulating soy milk with a coagulant, such as calcium sulfate.
[c]Tempeh is an Indonesian cake-like food prepared by fermentation of soya beans.
[d]Miso is traditional Japanese seasoning produced by fermenting rice and soybeans.

(Pueraria lobata, Fabaceae) native to southern Japan and south east China and possibly other species in the genus *Pueraria*. In traditional Chinese medicine, kudzu is considered one of the 50 fundamental herbs. It is used to treat tinnitus, vertigo and Wei syndrome (superficial heat close to the surface).

Prenylflavonoids

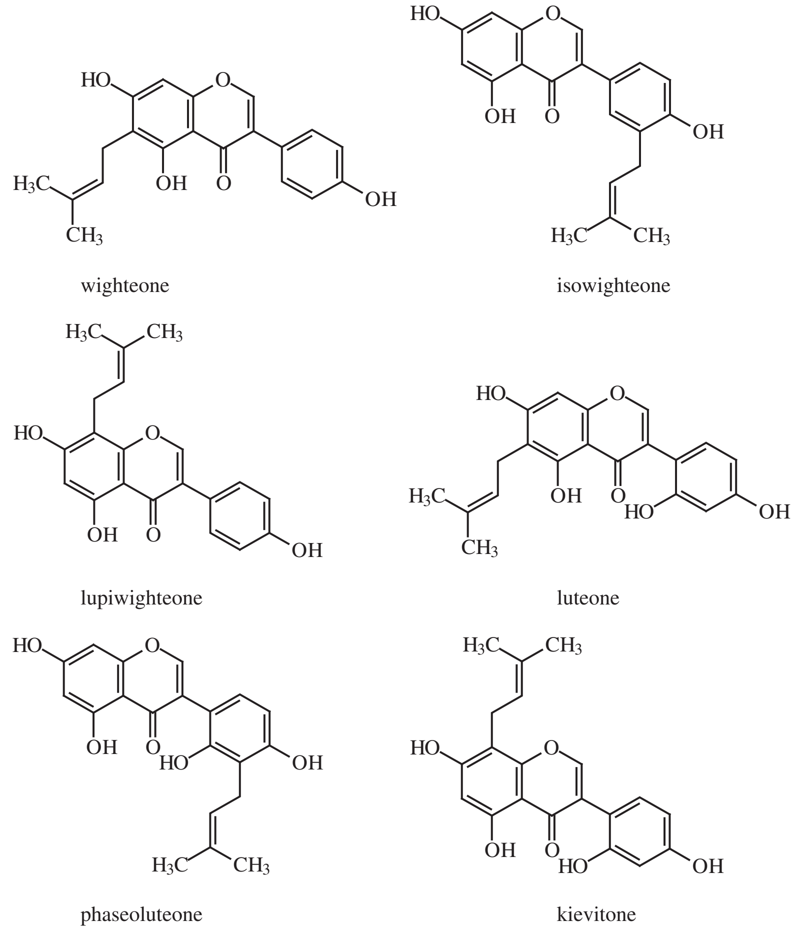
Legume plants also contain many lipophilic prenylated isoflavones (**10-97**), which are, in comparison with the original isoflavones, more efficient defence substances against pathogenic ilicitors (microorganisms) and animal pests (phytoalexins). They arise by prenylation of isoflavone precursors by dimethylallyl diphosphate.

Prenylated isoflavones are related to prenylated flavonoids (chalcones and flavanones or dihydroflavones) located in mature female cones of hops (*Humulus lupulus*, Cannabinaceae), which exhibit oestrogenic and anticarcinogenic effects. Their amount depends on the weather conditions and the hops variety. The most common compound is a chalcone xanthohumol (**10-98**), which does not exhibit oestrogenic activity. Its content in hop cones is around 1%. Xanthohumol is accompanied by the flavanone isoxanthohumol, which arises from xanthohumol by conversion in an acidic media during heating. Isoxanthohumol is therefore the main prenylated flavonoid in beer. At concentrations of about 10 to 100 times lower, prenylated flavonoids are accompanied by chalcone demethylxanthohumol, which is a precursor of most hop prenylflavonoids. The main oestrogen is flavanone 8-prenylnaringenin accompanied by 6-prenylnaringenin (**10-98**) and other compounds. Concentrations of 8- prenylnaringenin in various beer brands range from <1.6 μg/l in non-alcoholic beers to 138.5 μg/l in stouts. 8-Prenylnaringenin also results from isoxanthohumol by the action of bacteria in the colon or liver (cytochrome P450 enzymes). Chalcone xanthogalenol (**10-98**) has only been detected in the US and East Asian hops varieties.

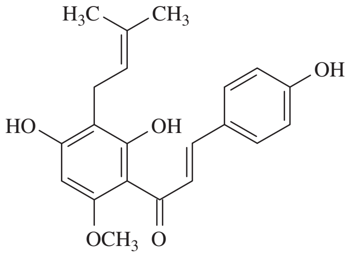
Pterocarpans

Pterocarpans are a group of modified isoflavonoids occurring in small amounts in legume plants (Fabaceae) and in several other families. They typically act as phytoalexins, protecting plants against pathogens. Unlike most of the isoflavones, pterocarpans are synthesised in response to biotic and abiotic stress factors. Their important precursors are the isoflavones daidzein and formononetin.

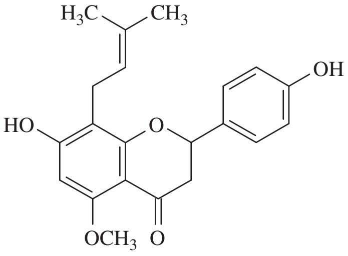
In germinated soybeans and other legumes occurs, together with isoflavone formononetin, as the major oestrogen coumestrol (**10-99**), which is present in an amount ranging from 0.05 to 0.2 mg/kg. During germination, its concentration increases about 70–150 times. The highest amount of coumestrol occurs in hulls



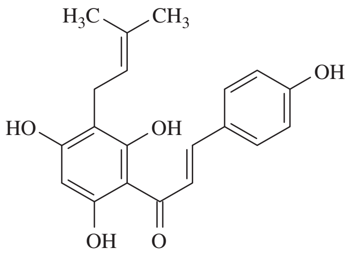
10-97, prenylated isoflavones of legumes



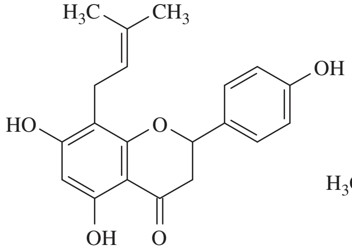
xanthohumol



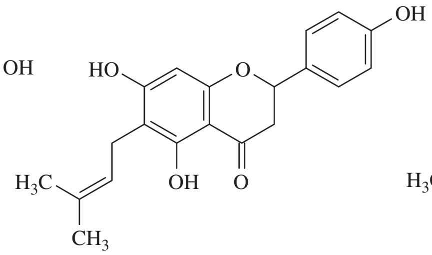
isoxanthohumol



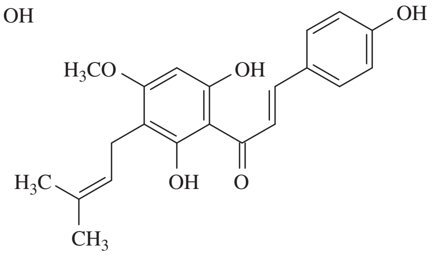
demethylxanthohumol



8-prenylnaringenin

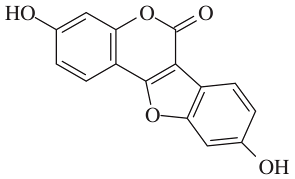


6-prenylnaringenin

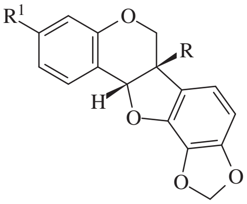


xanthogalenol

10-98, main prenylated flavonoids of hops

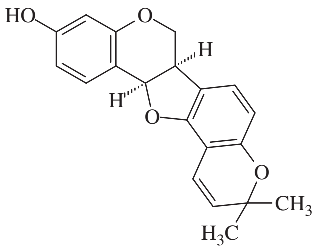


10-99, coumoestrol

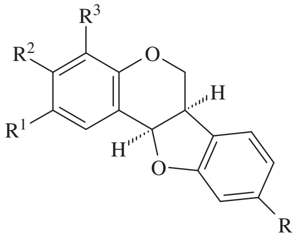


10-101, (+)-maackiain, R = H, R^1 = OH
(+)-pisatin, R = OH, R^1 = OCH_3

of beans. Oestrogenic activity of coumestrol is 30–40 times higher than activities of isoflavones. Small quantities of other related compounds are present, such as medicarpin, lucernol, sativol (**10-100**) and other pterocarpans. Coumestrol is the main oestrogen of most forage crops (legumes), such as alfalfa (*Medicago sativa*), where it is accompanied by medicarpin (**10-100**). Maackiain (**10-101**) occurs in chickpea (*Cicer arietinum*), pisatin (**10-101**) in pea (*Pisum sativum*), phaseolin (**10-102**) in common beans (*Phaseolus vulgaris*) and glyceollins (**10-103**) in soya beans (*Glycine max*).



10-102, (−)-phaseolin

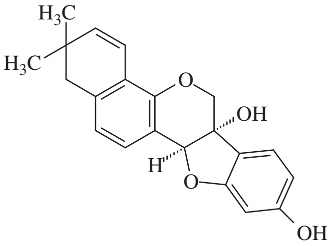


10-100, (−)-medicarpin, R = OCH_3, R^1 = H, R^2 = OH, R^3 = H
(−)-lucernol, R = OH, R^1 = OH, R^2 = OH, R^3 = H
(−)-sativol, R = OH, R^1 = H, R^2 = OCH_3, R^3 = OH

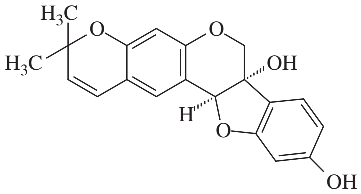
According to the structure, pterocarpans are divided into simple pterocarpans (an example is medicarpin with a methoxyl group and maackiain with a methylenedioxy group), pterocarpens (such as coumestrol) and 6-hydroxypterocarpans (such as pisatin), some of which, such as glyceollin III (**10-103**) with a prenylated substituent on the furan ring, have a more complex structure.

Lignans

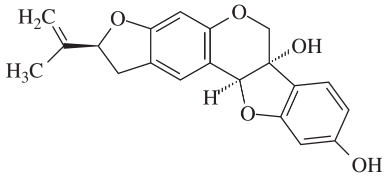
Lignans are a group of plant phenolic compounds, classified as phenylpropanoids, which are derived from coniferyl alcohol and



glyceollin I



glyceollin II



glyceollin III

10-103, glyceollins

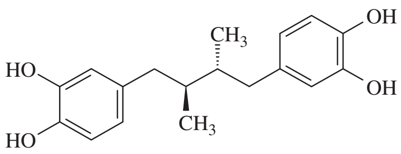
other cinnamyl alcohols. Lignans are dimers of type $(C_6–C_3)_2$ formed by association of two phenylpropane units on the central carbon atom of the side chain by bond C-8/C-8′ with the formation of a $C_6–C_3–C_3–C_6$ skeleton. The polymer of phenylpropane units known as lignin (see Section 4.5.1.7.1) has an analogous structure. Dimers of type $C_6–C_3–C_6–C_3$ are called **neolignans**. Together with the polymeric lignin, lignans are the most widespread plant components, which have a function in protecting the plant against pathogens. They also have a variety of other biological effects, as they additionally act as phytooestrogens, antioxidants and anticarcinogenic agents.

Several hundred lignans have been identified. Depending on the degree of skeleton oxidation, several types of lignans are distinguished, of which four types of linear lignans are of the greatest importance in food materials (**10-104**):

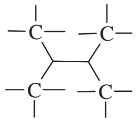
- lignans, butane derivatives also called **diarylbutanoids**,
- derivatives of butanolide called **lignanolides**,
- derivatives of tetrahydrofuran called **monoepoxylignans**,
- furofuran lignans called **bisepoxylignans**, which are derivatives of 3,7-dioxabicyclo[3.3.0]octane.

Another cyclisation providing a new bond C-7/C-6′′ yields **cyclolignans** (cyclic lignans), which can be considered derivatives of naphthalene or tetrahydronaphthalene (**10-104**).

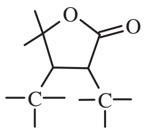
The longest known lignan of diarylbutanoid type is nordihydroguaiaretic acid, 4,4’-(2,3-dimethylbutane-1,4-diyl)dibenzene-1,2-diol, abbreviated NDGA (**10-105**), the biosynthesis of which is based on caffeoyl alcohol. In the 1950s and 1960s NDGA was used as an antioxidant, initially in the form of a resinous product obtained from the North American evergreen creosote bush (*Larrea tridentata*, Zygophyllaceae) and later as a synthetic substance.



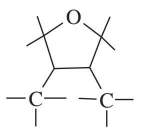
10-105, nordihydroguaiaretic acid



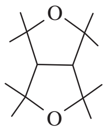
diarylbutanoids



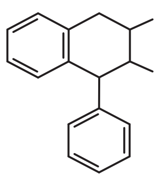
lignanolides



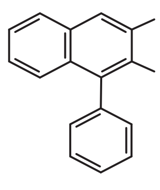
monoepoxylignans



bisepoxylignans



tetrahydronaphthalene structure

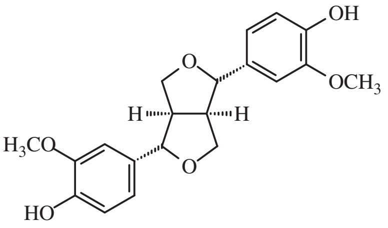


naphthalene structure

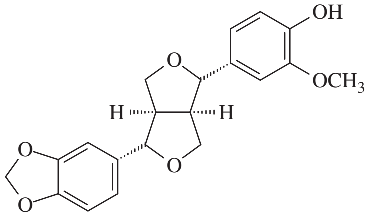
10-104, basic structures of lignans and cyclolignans

The antioxidant properties of NDGA are similar to the properties of gallates, but because of its adverse toxicological effects it has no practical significance.

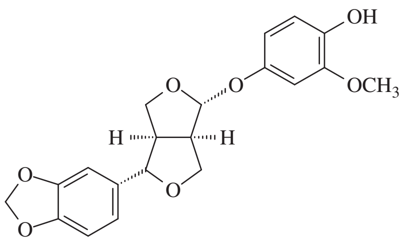
Based on coniferyl alcohol, the bisepoxylignan (+)-pinoresinol (**10-106**) is a precursor of many other bisepoxylignans, such as (+)-piperitol (**10-107**), (+)-sesamolinol (**10-108**), (+)-sesamin (**10-109**) and (+)-sesamolin (Figure 10.11). Sesamin is a fairly widespread compound in higher plants, found in large quantities in sesame oil obtained from the seeds of Indian sesame (*Sesamum indicum*, Pedaliaceae), which is highly resistant to oxidation. The main components of sesame seed are sesamin (0.1–10 g/kg) and sesamolin (0–10 g/kg). In addition to abundant sesamin and sesamolin, several minor furofuran lignans are also found in sesame seeds, such as (+)-pinoresinol, (+)-piperitol, (+)-sesamolinol, (−)-episesamin, also known as (−)-asarinin (**10-110**), along with other substances. Hydroxysubstituted lignans and compounds with an opened 1,3-dioxol ring are present as glycosides (β-gentiobiosides, β-sophorosides, di- and tri-β-glucosides). Water-soluble lignan glycosides are, for example sesaminol diglucoside, sesaminol-2′-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, and sesaminol triglucoside, sesaminol-2′-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside) (**10-111**).



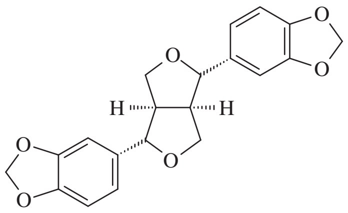
10-106, (+)-pinoresinol



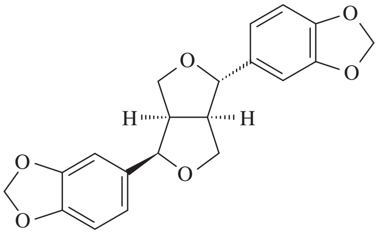
10-107, (+)-piperitol



10-108, (+)-sesamolinol



10-109, (+)-sesamin



10-110, (−)-asarinin

Sesamin, in addition to a significant antioxidant effect, also shows an insecticidal effect. The actual antioxidant (having an effect comparable to BHA and BHT) is sesamol (Figure 10.11), which is present in small amounts in seeds, but which arises in larger quantities by hydrolysis of sesamoline during sesame oil refining (especially in bleaching) and during thermal operations (such as frying). Sesamol is very easily oxidised in the presence of Fe ions and produces cytotoxic oxidation products, which are structurally oligomeric compounds of sesamol (**10-112**). Oestrogenic bisepoxylignan (a precursor of enterolactone) is (−)-asarinin (**10-110**), occurring also in some medicinal plants, for example in *Asiasarum sieboldi* (syn. *Asarum sieboldi*) of the birthwort family

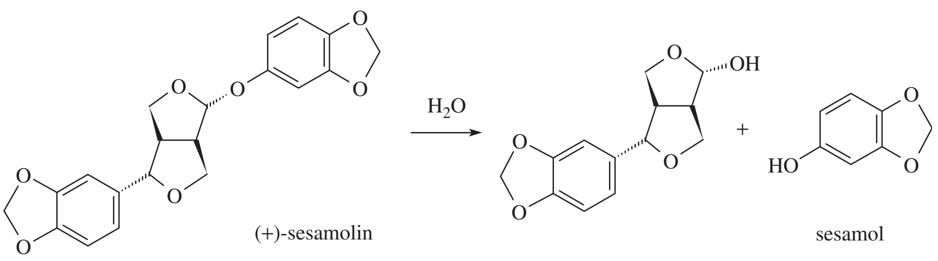
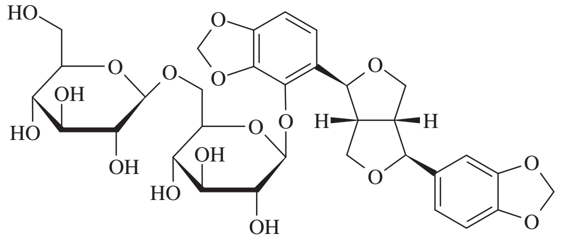
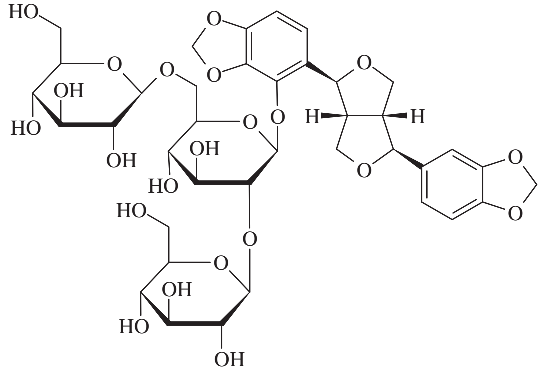


Figure 10.11 Formation of sesamol from sesamolin.



sesaminol diglucoside



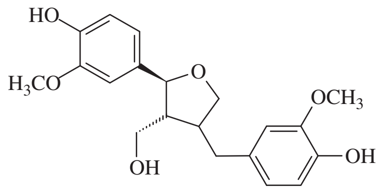
sesaminol triglucoside

10-111, sesaminol glucosides

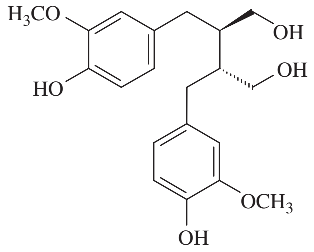
Aristolochiaceae, which is used in cosmetic skin conditioning preparations.

(+)-Pinoresinol (furofuran) is also a precursor of (+)-lariciresinol (benzylaryltetrahydrofuran, **10-113**), (−)-seco-isolariciresinol (dibenzylbutane, **10-114**) and (−)-matairesinol (dibenzylbutyrolactone, **10-115**), which are intermediates in the biosynthesis of cyclolignans derived from aryltetrahydronaphthalene. Isomerisation of (+)-pinoresinol gives (−)-pinoresinol (**10-116**), which is analogously the precursor of (−)-lariciresinol (**10-117**) and (+)-secoisolariciresinol (**10-118**).

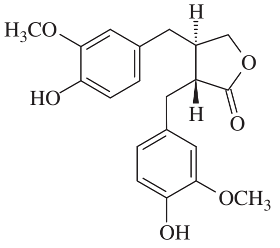
The most important oestrogenic compounds are (−)-matairesinol, (3*R*,4*R*)-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl] oxolan-2-one, and (−)-secoisolariciresinol, (2*R*,3*R*)-2,3-bis[(4-hydroxy-3-methoxyphenyl)methyl]butane-1,4-diol, which are found in plant materials in the form of glycosides. For example, (−)-mataresinol may occur as 4-*O*-β-D-glucoside or 4,4′-di-*O*-β-D-glucoside (**10-119**), while the principal lignan precursor found in flaxseed is (−)-secoisolariciresinol 2,3-di-*O*-β-D-glucoside (**10-120**). In foods, these lignans occur as the main components of whole grain cereal products, in rice, legumes, nuts and various other seeds, and also in vegetables and fruits (Table 10.17).



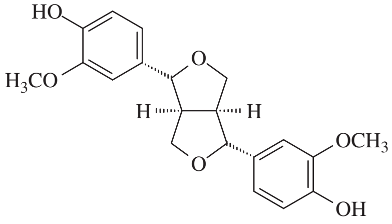
10-113, (+)-lariciresinol



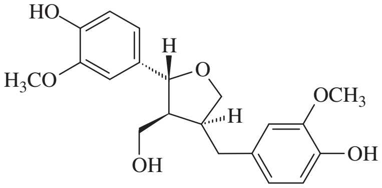
10-114, (−)-secoisolariciresinol



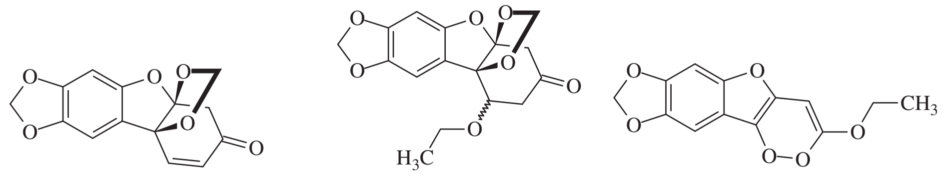
10-115, (−)-matairesinol



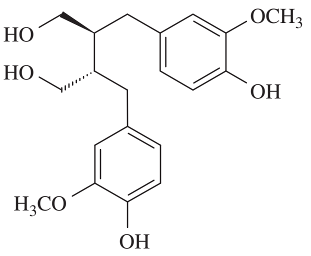
10-116, (−)-pinoresinol



10-117, (−)-lariciresinol

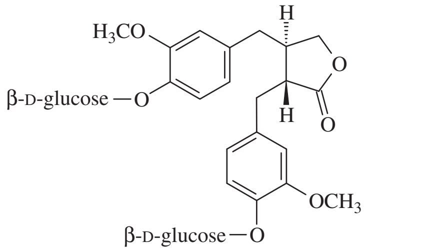


10-112, sesamol oxidation products

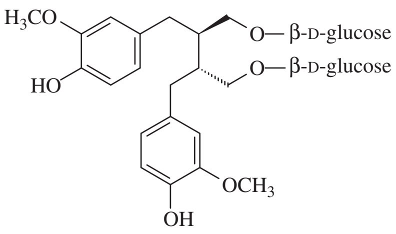


10-118, (+)-secoisolariciresinol

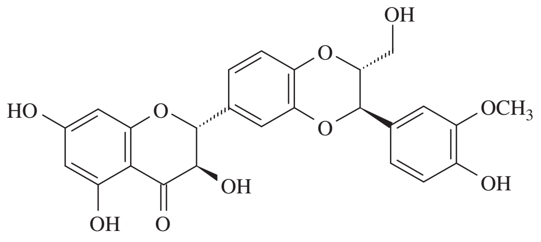
Related to lignans are **flavonolignans**, which do not exhibit oestrogenic activity. Their biosynthesis is based on flavonoids (such as dihydroflavonol taxifolin or dihydroquercetin) and phenylpropanoids, usually coniferyl alcohol. Examples of flavonolignans are components of the pericarp of milk thistle (*Silybum marianum*, Asteraceae) called silymarin, of which 70–80% are structurally related flavonolignans. The main component of the silymarin complex is silybin (also known as silibinin), which is a mixture of diastereoisomers A (**10-121**) and B (**10-122**) occurring in the ratio of 1 : 1. Milk thistle is recommended for indigestion, as a



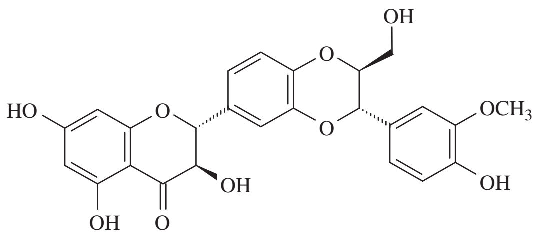
10-119, (–)-matairesinol 4,4′-di-*O*-β-D-glucoside



10-120, (–)-secolariciresinol 2,3-di-*O*-β-D-glucoside



10-121, silybin A



10-122, silybin B

Table 10.17 Lignans in selected foods.

Product	Water (%)	Content (μg/kg)				
		Pinoresinol	Lariciresinol	Secoisolariciresinol	Matairesinol	Total
Linseed	7.0	332	304	29 420	55.3	30 110
Sesame seed	4.7	2 933	947	6.6	48.1	3 935
Peanuts	6.5	0	4.1	5.3	0	9.4
Broccoli	89	56.8	21.2	0.8	0	78.7
Carrots	92	1.9	8.0	9.3	0	17.1
Onion	90	0	1.9	1.8	0	3.6
Spinach	94	1.2	6.8	0.2	0	8.2
Tomatoes	94	1.4	4.2	0.2	0	5.8
Potatoes	80	0	1.7	0.2	0	2.0
Oranges	86	2.4	4.7	0.5	0.2	7.8
Peaches	80	18.6	8.0	2.7	0	29.3
Olive oil	0.0	24.3	0.4	0	0	24.8

supportive agent in chronic hepatitis therapy and liver cirrhosis, and in gallbladder problems and for loss of appetite.

Other phytooestrogens

Many cereals accumulate hydroxamic acids derived from the lactam 2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one that are one of the most important chemical barriers against herbivores and have been shown to be inhibitory to several plant pathogenic fungi and bacteria. For example, these benzoxazinoid hydroxamic acids are involved in the defense of maize against various lepidopteran pests, most notably the European corn borer, in the defense of cereals against various aphid species and in allelopathy affecting the growth of weeds associated with rye and wheat crops. The biosynthetic pathway to benzoxazinoid hydroxamic acids differs from tryptophan biosynthesis at indole-3-glycerol phosphate, which is converted into indole and indolin-2-one (Figure 10.12). Subsequently four oxygen atoms are enzymatically introduced into the indolin-2-one moiety via 3-hydroxyindolin-2-one, (*R*)-2-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (known under the acronym HBOA) and (*R*)-2,4-dihydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIBOA). After glucosylation, the glucoside is further modified by hydroxylation to (*R*)-2,4,7-trihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (TRIBOA glucoside) and *O*-methylation at C-7 to form (*R*)-2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one (DIMBOA glucoside). The predominant benzoxazinoids are DIBOA and its 7-methoxy derivative DIMBOA, which are stored as glucosides in vacuoles. Upon tissue damage, specific glucosidases present in the plastids come into contact with the glucosides and bioactive aglycones, 3*H*-benzoxazol-2-ones (2,3-dihydro-1,3-benzoxazol-2-ones), are produced. Young plants of wheat, maize, rice and other cereals contain mainly 3*H*-1,3-benzoxazol-2-one (BOA) and 7-methoxy-3*H*-benzoxazol-2-one (MBOA) that do not have oestrogenic activities, but stimulate the secretion of oestradiol from ovarian follicles, showing signs of oestrogen activities. Lactams, the corresponding hydroxamic acids and derived benzoxazolones are collectively known as **benzoxazinoids**. Various benzoxazinoids also occur in bread, where the main components are DIBOA and MBOA.

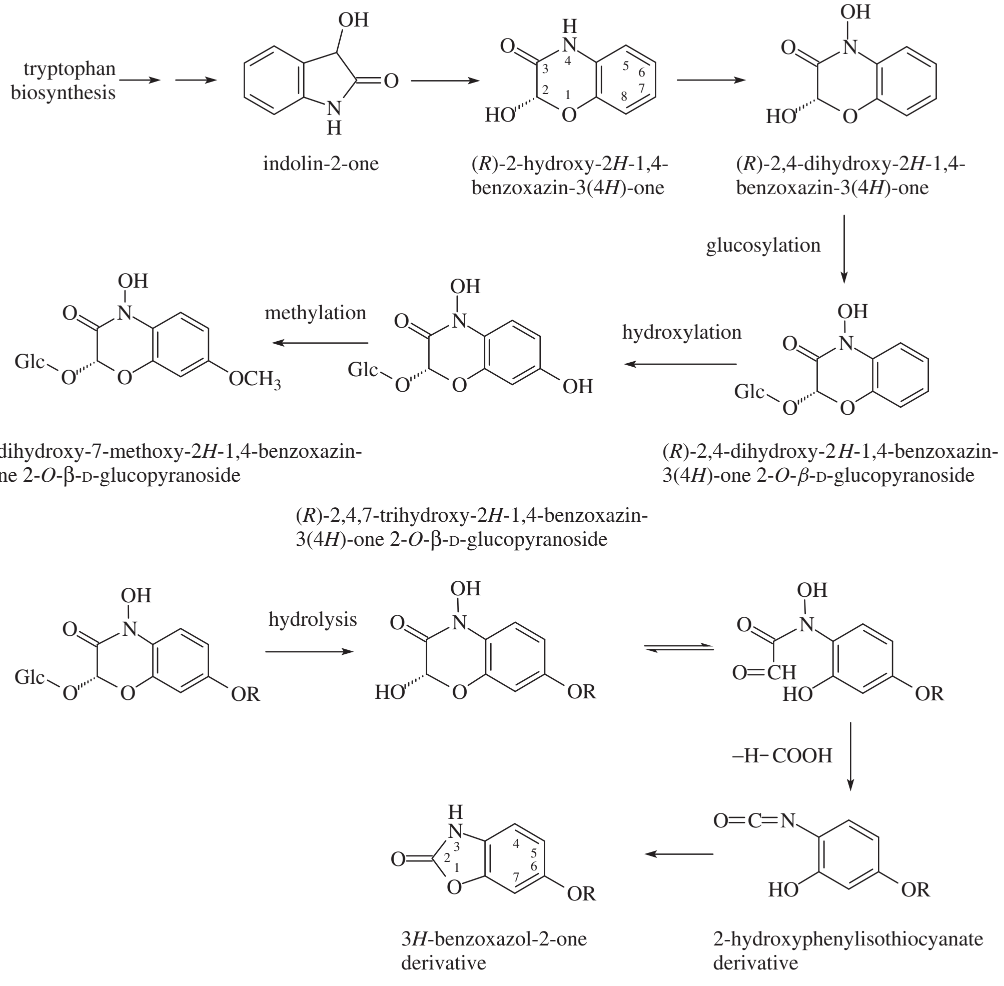


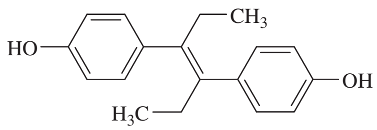
Figure 10.12 Biosynthesis and transformation of benzoxazinoid hydroxamic acids.

Mycooestrogens

The most effective mycooestrogen from the group of resorcinic acid lactones is (*E*)-6-(10-hydroxy-6-oxoundec-1-en-1-yl)-2,4-dihydroxybenzoic acid lactone, trivially known as zearalenone or F-2 toxin. Zearalenone is produced by fungi of the genus *Fusarium* (see Section 12.3.1.2.1).

Xenooestrogens

Comparable, or even higher, oestrogenic activity than oestradiol is exhibited by synthetic stilbene (*E*)-stilboestrol (*trans*-diethylstilboestrol, **10-123**), which was originally used in the fattening of cattle and other livestock, because it accelerated their growth. In human medicine, *trans*-diethylstilbestrol was used to prevent miscarriages, but because of its carcinogenic effects, the compound is no longer used.



10-123, (*E*)-diethylstilboestrol

Oestrogenic activity has been demonstrated in a number of other anthropogenic substances. An example is bisphenol A (see Section 12.9.2), a monomer of polycarbonate and epoxy resins, which can be leached from lacquered plates for cans or dental compositions and seals. Oestrogenic activity is also exhibited by some halogenated pesticides, polychlorinated biphenyls and certain of their hydroxylated metabolites, phthalates (used as plastic softeners), veterinary pharmaceuticals and some other xenobiotics, which are referred to in Chapter 12.

Reactions and changes

Isoflavones are transferred virtually unchanged from soya beans into flour. The loss caused by leaching during soaking of soya beans is about 11%. Cooking results in about a 50% loss of the original content. About 40% of isoflavones are lost during extraction with alkaline agents during production of protein isolates. Acid hydrolysates of soybean meal contain only isoflavone aglycones formed by hydrolysis of glycosides.

Biological effects

Oestrogens are both useful and harmful for the organism. An endogenous oestrogen of mammals is oestradiol, which is essential for normal development and reproduction of the organism, and also affects other important processes related to the immune system and central nervous system. At the same time, however, oestradiol is associated with cancers of the mammary glands, rectum and probably also the prostate.

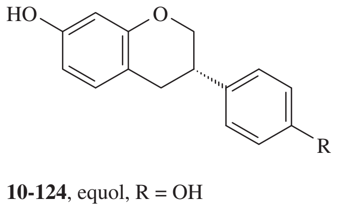
The effects of other oestrogens are still under discussion. Phytooestrogens show several orders of magnitude lower oestrogenic activity than oestradiol (Table 10.18), but given the quantities in which they are consumed, they may cause male cattle infertility and abortions in female cattle.

Table 10.18 Relative activity of selected oestrogens.

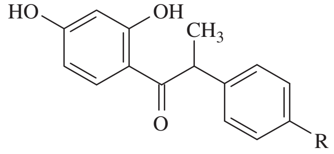
Oestrogen	Relative activity (%)
(*E*)-Diethylstiboestrol	100
Oestron	6.9
Coumoestrol	0.035
Genistein	0.001
Daidzein	0.000 75
Biochanin A	0.000 46
Formononetin	0.000 26

In women consuming food rich in phytooestrogens, irregularities of the menstrual cycle were observed. On the other hand, in a population with a high intake of soy isoflavones (such as in some Asian countries), lower incidence of breast cancer in women and of prostate cancer in men was reported. The activity of phytooestrogens is generally an additive property. If the level of endogenous (steroid) oestrogens is low (typically in animals), phytooestrogens bind together with them to the receptors. If the level of steroid oestrogens is high (usually in women), phytooestrogens act antagonistically (as antioestrogens).

Some isoflavones do not exhibit oestrogenic activity themselves. Formononetin is not an oestrogenic substance; however, in the rumen of ruminant animals (as well as in silage feed and the digestive tract of humans, as it also occurs in the urine of women after consumption of soya products) it is transformed by bacteria after the hydrolysis of sugar to aglycone and further, via daidzein, to the oestrogenic compound equol (7,4′-dihydroxyisoflavan, **10-124**), which is the main product of this transformation. Byproducts of formononetin (**10-125**) transformations are angolensin and 4′-*O*-methylequol (**10-124**), from which equol arises by a side-path reaction. Equol is partially transformed into *O*-demethylangolensin



10-124, equol, R = OH
4′-*O*-methylequol, R = OCH_3

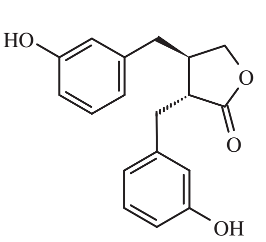


10-125, *O*-demethylangolensin, R = OH
angolensin, R = OCH_3

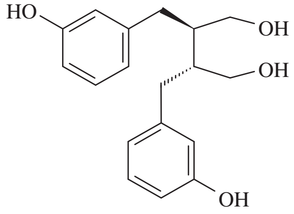
(**10-125**). Equol represents about 70% of formononetin degradation products, *O*-demethylangolensin 5–20% and minor products are of 4′-*O*-methylequol and angolensin.

For example, the infertility syndrome called clover disease of sheep, occurring in the 1940s in Western Australia, was caused by equol. The disease was manifested by a decrease of sheep gestation, frequent abortions and postnatal complications. Similar syndromes have been found in other animals.

Biochanin A is degraded to genistein and further to dihydrogenistein, from which 4-ethylphenol and other simple products arise. The bacterial degradation products of matairesinol in the digestive tract are enterolactone (**10-126**) and lariciresinol. Secoisolariciresinol gives rise to enterodiol (**10-127**), which can be oxidised to the enterolactone. These compounds are excreted in the urine.



10-126, enterolactone



10-127, enterodiol

The result of exposure to anthropogenic oestrogens in men is believed to be lower sperm quality, and in women there is a higher incidence of lung cancer. Oestrogens may also cause anomalies in wildlife (such as feminisation of male fish). These findings are still mainly speculative due to the small amounts of consumed xenooestrogens, compared with the much higher amount of oestrogens produced endogenously and with the amount of consumed phytooestrogens.

In addition to oestrogenic activity, isoflavones also exhibit relatively high antioxidant activity, which requires the presence of two hydroxyl groups (at C-7 and C-4′) in the molecule, as found in daidzein, which is therefore the most active antioxidant. The activity of aglycones is higher than the activity of glycosides, but, generally, isoflavones have lower antioxidant activity than the corresponding flavones. Isoflavones are also major contributors to the astringent and bitter taste of soya beans.

10.3.3.5.2 Phototoxic compounds

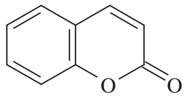
Phototoxicity and other toxic effects are exhibited by a group of phenolic compounds called **coumarins**. The primary photosensitisers are furanocoumarins and some plant pigments, such as hypericin of St. John's wort (*Hypericum perforatum*, Hypericaceae) and fagopyrin (**9-174**) of common buckwheat (*Fagopyrum esculentum*, Polygonaceae).

Structure, nomenclature and occurrence

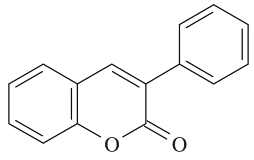
Coumarins More than 1000 coumarins occur in nature, of which about 300 compounds are simple coumarins. Coumarins occur most often as a mixture of 10–20 related compounds in about 100 families of plants, but especially in plants of the Rutaceae family, which includes various citruses, Moraceae, to which mulberries (*Morus* spp.) and figs (*Ficus* spp.) belong, Apiaceae, including many species of aromatic vegetables (carrots, parsley, parsnips and celery) and spices (caraway, cumin, coriander, dill, anise and fennel) and Asteraceae, which includes some medicinal herbs. Simple coumarins (including 2*H*-1-benzopyran-2-ones and 5,6-benzo-2-pyrones) are derived from γ-lactones of 2-hydroxycinnamic acids. The basic member of the homologous series of coumarins (**10-128**) is coumarin. Almost all other simple coumarins are non-volatile compounds hydroxylated at C-7. Other skeletal positions may also be hydroxylated and some hydroxyl groups are protected by methylation or isoprenylation. Coumarins also often occur as glycosides, which are accompanied by respective aglycones. Some coumarins act as blastocolins (inhibiting seed germination). Relatively widespread are different **furanocoumarins**, which are linear (6,7-furanocoumarins, also known as psoralen type furanocoumarins or psoralens) or angular compounds (7,8-furanocoumarins, angelicin type furanocoumarins or angelicins). Linear and angular **pyranocoumarins** are relatively rare. They are found mainly in plants of the Rutaceae family. I**socoumarins** (3,4-benzo-2-pyrones) are also rare, and 3-phenylcoumarins (**isoflavonoids**) and 4-phenylcoumarins (**neoflavonoids**) are less common.

Typical representatives of simple coumarins substituted at position C-6 or at positions C-6 and C-7 are umbelliferon (7-hydroxycoumarin), derived from 4-coumaric acid, and its 7-*O*-β-D-glucopyranoside skimmin, aesculetin (6,7-dihydroxycoumarin, formerly also known as cichorigenin), its 6-*O*-β-D-glucopyranoside aesculin (or aesculoside) and 7-*O*-β-D-glucopyranoside cichoriin, scopoletin (7-hydroxy-6-methoxycoumarin) and its 7-*O*-β-D-glucopyranoside scopolin and scoparon (6,7-dimethoxycoumarin, **10-129**).

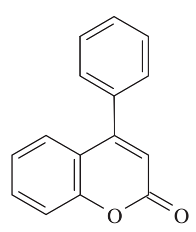
These coumarins are present in small amounts in some fruits and vegetables as phytoalexins. Aesculetin and scopoletin were found at a level of about 1 mg/kg in carrots, celery and other root vegetables and some fruits (such as apricots). The same compounds, aesculetin-6-*O*-β-D-glucoside (aesculin) and aesculetin 7-*O*-β-D-glucoside (cichoriin) also occur in the roots and buds of chicory, and together with other substances contribute to the bitter taste of this vegetable. The bark of horse chestnut (*Aesculus* spp., Hippocastanaceae) contains a large amount of aesculin. Scoparone occurs in citrus fruits exposed to UV radiation or significant temperature fluctuations. Hemiarin (7-methoxycoumarin) and ajapin (6,7-methylenedioxycoumarin, **10-130**) are found in tubers of Jerusalem artichokes (*Helianthus tuberosus*, Asteraceae).



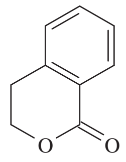
coumarin
(chromen-2-ones)



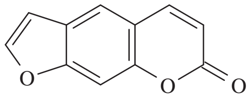
3-phenylcoumarin
(isoflavonoids)



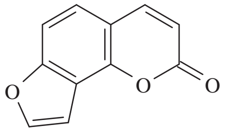
4-phenylcoumarin
(neoflavonoids)



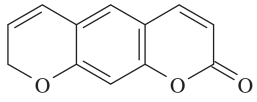
isocoumarin



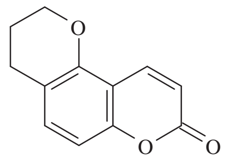
6,7-furanocoumarin
(linear furanocoumarins)



7,8-furanocoumarin
(angular furanocoumarins)

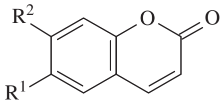


linear pyranocoumarin

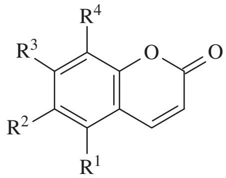


angular pyranocoumarin

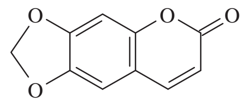
10-128, coumarins and related compounds



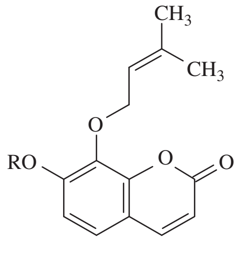
10-129, important coumarins
umbelliferon, $R^1 = H$, $R^2 = OH$
aeculetin, $R = R^2 = OH$
hemiarin, $R^1 = H$, $R^2 = OCH_3$
scopoletin, $R^1 = OCH_3$, $R^2 = OH$
scoparon, $R^1 = R^2 = OCH_3$



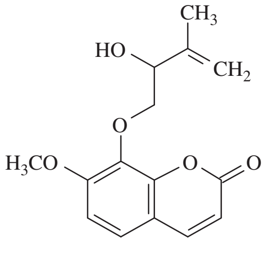
10-131, other important coumarins
daphnetin, $R^1 = R^2 = H$, $R^3 = R^4 = OH$
limettin, $R^1 = R^3 = OCH_3$, $R^2 = R^4 = H$
fraxidin, $R^1 = H$, $R^2 = R^3 = OCH_3$, $R^4 = OH$



10-130, ajapin



10-132, isoprenoid coumarins
osthenol, R = OH
osthol, R = OCH_3



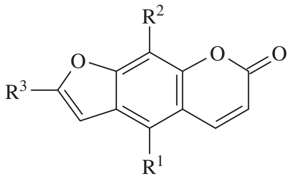
auraptenol

Examples of coumarins substituted at other positions of the benzene ring are daphnetin (7,8-dihydroxycoumarin), limettin (5,7-dimethoxycoumarin) and fraxidin (6,7-dimethoxy-8-hydroxycoumarin, **10-131**). Limettin, for example, occurs in citrus fruits, dafnetin-8-*O*-β-ᴅ-glucoside, known as daphnin, is present in higher quantities in the bark of daphne (*Daphne mezereum*, Thymelaeaceae), commonly known as mezereon, and fraxidin-8-*O*-β-ᴅ-glucoside or fraxin occurs together with related coumarins in the bark of common ash (*Fraxinus excelsior*, Oleaceae).

An example of coumarins with isoprenoid substituents is osthenol, 7-hydroxy-8-(2-hydroxy-3-methyl-3-en-1-yl)coumarin (**10-132**), which together with osthol, 7-methoxy-8-(3-methyl-2-en-1-yl)coumarin and auraptenol, 7-methoxy-8-(2-hydroxy-3-methyl-3-en-1-yl)coumarin, occurs in citrus fruits. In common with many other coumarins, it is found in garden angelica (*Angelica archangelica*, Apiaceae) and other plants.

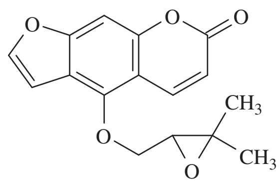
Furanocoumarins These are frequently found in plants and are synthesised from umbelliferon. There are two basic types of furoanocoumarins:

- linear coumarins or 6,7-furanocoumarins, or psoralen type coumarins or psoralens
- angular coumarins or 7,8-furanocoumarins or angelicin type coumarins or angelicins.



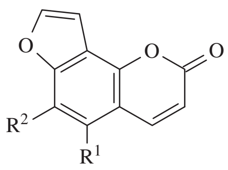
10-133, main psoralen type furanocoumarins
psoralen, $R^1 = R^2 = R^3 = H$
bergaptol, $R^1 = OH$, $R^2 = R^3 = H$
bergapten, $R^1 = OCH_3$, $R^2 = R^3 = H$
xanthotoxin, $R^1 = R^3 = H$, $R^2 = OCH_3$
isopimpinellin, $R^1 = R^2 = OCH_3$, $R^3 = H$
trioxsalen, $R^1 = R^2 = R^3 = CH_3$
imperatorin, $R^1 = R^3 =$, $R^2 = OCH_2CH{=}C(CH_3)_2$
isoimperatorin, $R^1 = OCH_2CH{=}C(CH_3)_2$, $R^2 = R^3 = H$
fellopterin, $R = OCH_3$, $R = OCH_2CH{=}C(CH_3)_2$, $R^3 = H$
bergamottin, $R^1 = OCH_2CH{=}C(CH_3)(CH_2)_2CH{=}C(CH_3)_2$, $R^2 = R^3 = H$

Common linear hydroxy and methoxyl substituted furanocoumarins (**10-133**) include psoralen, bergaptol (5-hydroxypsoralen), bergapten (5-methoxypsoralen), xanthotoxin (8-methoxypsoralen) and isopimpinellin (5,8-dimethoxypsoralen). Some other linear furanocoumarins, such 8-(3-methyl-2-en-1-yloxy)psoralen (imperatorin), 5-(3-methyl-2-en-1-yloxy)psoralen (isoimperatorin), 5-methoxy-8-(3-methyl-2-en-1-yloxy)psoralen (fellopterin) and 5-(3,7-dimethyl-2,6-dien-1-yloxy)psoralen or 5-geranoxypsoralen (bergamottin) have an alkenoxyl isoprenoid chain as do the simple coumarins osthol and auraptenol. These compounds are found mainly in citrus essential oils and root vegetables. Psoralen and bergapten are also the main coumarins of fig tree leaves. A more complex structure is oxypeucedanin, 5-(3,3-dimethyloxiranylmethoxy)psoralen (**10-134**), which occurs as (*R*)- and (*S*)-isomers in parsley and garden angelica, for example.

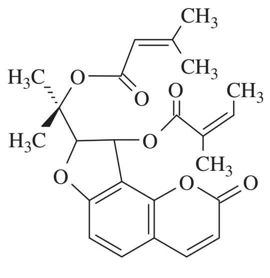


10-134, oxypeucedanin

Angular type furanocoumarins (**10-135**) include angelicin, isobergapten (5-methoxyangelicin) sphondin (6-methoxyangelicin) and pimpinellin (5,6-dimethoxyangelicin). In small amounts, these compounds accompany linear furanocoumarins. An example of a complex furanocoumarin of the angelicin type is archangelicin (**10-136**), which occurs in garden angelica.



10-135, angelicin type furanocoumarins
angelicin, $R^1 = H$, $R^2 = H$
isobergapten, $R^1 = OCH_3$, $R^2 = H$
sphondin, $R^1 = H$, $R^2 = OCH_3$
pimpinellin, $R^1 = OCH_3$, $R^2 = OCH_3$



10-136, archangelicin

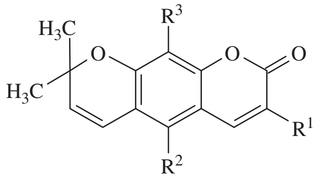
From a hygienic–toxicological point of view, psoralen type furanocoumarins are important in foods, and their major sources are mainly citrus fruits and root vegetables. Juice from the leaves of the common fig tree (*Ficus carica*, Moraceae) contains 2000 mg/l of psoralen and 500 mg/l of bergapten., These compounds are found in some spices in a small amount (Table 10.19).

The concentration of coumarins in plants is dependent on a number of factors. In healthy plants, their content varies in their different parts and greatly depends on climatic conditions. For example, in celery (*Apium graveolens* var. *dulce*, Apiaceae), whose leaves are eaten raw as a salad and cooked as a vegetable, the content of furanocoumarins in older outer leaves is 50 mg/kg, in the inner leaves 8 mg/kg and in the small central leaves 4 mg/kg fresh matter. The content in leaf petioles is about 0.2 mg/kg fresh matter. The amount of furanocoumarins is also low in the root, where it reaches only 1.1 mg/kg. A similar amount of furanocoumarins is found in the tuberous thick base of stems of celeriac (*A. graviolens*, var. *rapaceum*), which is cooked and eaten as a salad vegetable. High levels of furanocoumarins, however, appear as a result of different stress factors, such as attacks of microorganisms, exposure to UV radiation, storage at low temperatures, mechanical damage and other stress factors. For example, the attack of the fungus *Sclerotinia sclerotiorum* on the celery root results in a significant increase in the concentrations of these compounds (up to 44 mg/kg from about 2 mg/kg).

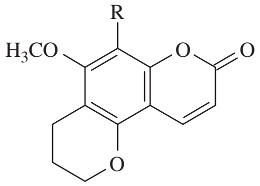
Pyranocoumarins Also found in plants, but in small amounts, are pyranocoumarins, of both linear (**10-137**) and angular types (**10-138**). Common linear pyranocoumarins are present mainly in plants of the Rutaceae family, and thus also in citrus fruits and,

Table 10.19 Furanocoumarins and pyranocoumarins in major food materials.

Material	Content of furanocoumarins (mg/kg)
Orange essentials oil	Bergaptol (0.5)
Lemon essentials oil	Bergapten (33)
Grapefruit essentials oil	Bergapten (120)
Carrot (root)	Bergapten (0.01), xanthotoxin (0.01)
Parsley (root)	Oxypeucedanin (26), bergapten (12), isoimperatorin (5.6), psoralen (1.4), Imperatorin (1.4), xanthotoxin (0.1), graveolon
Parsnips (root)	Xanthotoxin (48), angelicin (47), bergapten (7), psoralen (7)
Celery (root)	Bergapten (0.6), xanthotoxin (0.5), psoralen (0.04)
Celery (stalks)	Xanthotoxin (7.2), psoralen (1.0), bergapten (0.6)
Anise, coriander, caraway (seeds)	Xanthotoxin (0.01), bergapten (0.01)

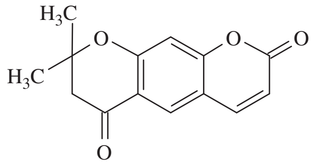


10-137, important linear pyranocoumarins
xanthiletin, $R^1 = H$, $R^2 = H$, $R^3 = H$
xanthoxylethin, $R^1 = OCH_3$, $R^2 = H$, $R^3 = H$
lugangetin, $R^1 = OCH_3$, $R^2 = H$, $R^3 = H$
trachyphyllin, $R^1 = OH$, $R^2 = CH{=}CHCH(CH_3)_2$, $R^3 = H$



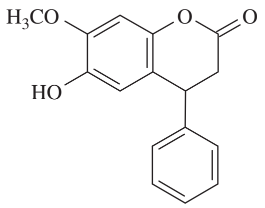
10-138, important angular pyranocoumarins
alloxanthoxyletin, R = H
dipetalin, $R = CH{=}CHCH(CH_3)_2$
aviceunin, $R = CH{=}CHC(CH_3){=}CH_2$

especially, in citrus essential oils extracted from the peel. A similar pyranocoumarin is graveolon (**10-139**), which is present in small amounts in the leaves and roots of parsley.



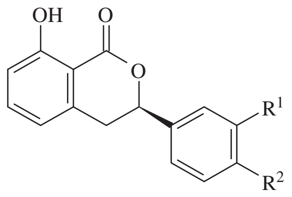
10-139, graveolon

3-Phenylcoumarins, 4-phenylcoumarins and isocoumarins A coumarin substituted at the C-3 phenyl group (having anisoflavone skeleton), known as santalin AC (6,7,3′-trihydroxy-2′,4′-dimethoxy-3-phenylcoumarin), is a minor component of red sandalwood colour (see Section 9.4.2.9). 4-Phenylcoumarins (also known as neoflavonoids), are found in trees of the genus *Dalbergia* (Fabaceae) grown in tropical regions of Asia and America. A typical representative is dalbergin (**10-140**).



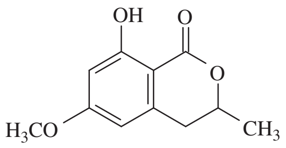
10-140, dalbergin

Furthermore, rarely found in plant materials are also isocoumarins (3,4-benzo-2-pyrones or 1*H*-2-benzopyran-1-ones), which are polyketides together with related stilbenes and coloured chalcones, but which are unlike other coumarins. Examples of dihydroisocoumarins are hydrangenol and phyllodulcin (**10-141**) located in the leaves of the large leaf hydrangea (*Hydrangea macrophylla*, Hydrangeaceae) and similar species. Phyllodulcin



10-141, hydrangenol, $R^1 = H$, $R^2 = OH$
phyllodulcin, $R^1 = OH$, $R^2 = OCH_3$

is used locally as a sweetener (see Section 11.3.2.1.3). A compound with oestrogenic activity is methoxymellein 6-(8-hydroxy-6-methoxy-3-methyl-3,4-dihydroisocoumarin, **10-142**). It occurs in carrots infected by a fungus and plant pathogen, *Ceratocystis fimbriata*, which gives the root a bitter taste.



10-142, 6-methoxymellein

Biological effects

Coumarins exhibit a wide variety of biological effects. They mainly act as vasodilatant agents and anticoagulants. For example, the synthetic dicoumarol warfarin is used in human medicine and as a rhodenticide (see Section 5.5.3). Furanocoumarins exhibit spasmolytic and vasodilatory effects, and coumarins and furanocoumarins have antibacterial and antifungal properties. For example, umbelliferon, scopoletin and furanocoumarins act as molluscocides (bergapten and isopimpinellin), they may have oestrogenic effects (some isocoumarins) and furanocoumarins can act as photosensitisers and thus exhibit phototoxic effects.

Photosensitisation caused by furanocoumarins is manifested by an increased sensitivity of unpigmented skin to sunlight (near-UV radiation of wavelength 400–300 nm, 3.10–4.13 eV, visible to birds, insects and fishes) and is associated with a higher incidence of skin cancer. The primary cause is binding of furanocoumarins to DNA (cycloaddition reactions with pyrimidine bases) and other macromolecular components of cells (such as fatty acids in lipoproteins) and the formation of free radicals activated by UV radiation. This disease is different from photo-dermatitis or sunburn, which is damage to the skin without the photosensitiser. Photosensitising diseases are, according to the origin of the photosensitiser and the way it gets into the peripheral circulation, distinguished into primary photosensitisation (the photosensitiser is an exogenous substance) and several others. Furanocoumarins and toxic pigments hypericin and phagopyrin (see Section 9.8.1.3.2) cause primary photosensitisation.

The toxicity of furanocoumarins occurring in foods (such as vegetables of the carrot family and citrus oils consumed in soft drinks, including flavoured teas) may be displayed in humans if the consumed amount ranges from 0.14 to 0.38 mg/kg body weight, which is out of the question in normal consumption of root vegetables. The total daily intake is estimated to be 1.3 mg. Photosensitisation caused by furanocoumarins may be a problem for workers handling celery or parsley leaves or coming into contact with the juice of fresh figs. It is reported that this so-called acute contact dermatitis appears after contact with celery leaves containing at least 18 mg/kg furancoumarins. Chronic dermatitis may appear in repeated contact with celery leaves even if the material contains about 7 mg/kg of furanocoumarins in fresh matter. In this respect, particularly infamous is the giant hogweed (*Heracleum mantegazzianum*, Apiaceae), native to Central Asia and introduced to some European countries, the United States and Canada as an ornamental plant, the sap of which causes severe dermatitis resulting in blisters, long-lasting scars, and blindness, if it comes in contact with the eyes.

Properties of furanocoumarins are also used therapeutically. Bergapten and xanthotoxin are used to treat psoriasis and the idiopathic depigmentation called vitiligo, which is caused by the formation of melanin pigment disorders associated with the activity of tyrosinase. After irradiation, psoralens act on the surrounding undamaged melanocytes, which are bigger, produce more melanin, and thus give the skin its normal colour.

10.3.3.6 Other natural antimicrobial compounds

Antimicrobial compounds, often showing many other biological effects, are synthesised and accumulated in plant tissues in response to an external stimulus. The external stimulae may be viruses or phytopathogenic organisms (bacteria and fungi), but may also include a variety of stress factors (such as UV radiation, cold, heavy metal ions, treatment with fungicides, herbicides and others). Compounds that are a permanent part of the plant tissues, synthesised by the plant at a constant rate and therefore always present in the tissues of the plant, are called **phytoanticipins**. They are produced by healthy plants from the beginning of growth and serve only as a passive defence against potential harmful factors. **Phytoalexins** are antimicrobial substances synthesised *de novo* by plants from phytoanticipins to combat infection by pathogens. Phytoalexins are also called **phytoncides**, **plant antibiotics** or **plant pesticides**, as they are toxic to pathogenic ilicitors (viruses and microorganisms) and animal pests (insects and higher animals). The distinction between phytoalexins and phytoanticipins is not always clear, as some compounds may be phytoalexins in one species and phytoanticipins in another species. In addition, the same substance may often serve as both phytoalexin and phytoanticipin. In general, the distinction between the two compounds depends on when they are produced, either before or after infection. The ability to synthesise and accumulate phytoalexins is documented in more than 200 species of plants belonging to about 40 genera and 20 families.

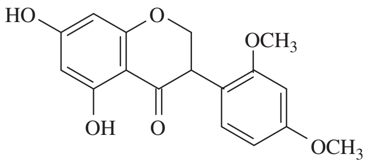
Examples of anticipins are glucosinolates in cruciferous (Brassicaceae) plants (see Section 10.3.3.4), which are enzymatically hydrolysed in disrupted tissue to form the antimicrobially active isothiocyanates. Another example is allicin in garlic, which is formed from the indifferent amino acid alliin by the activity of alliinase (see Section 8.2.9.2.2). Numerous phenolic compounds also exhibit antimicrobial activity in plant food materials, as do terpenoid substances, acetylene derivatives, heterocyclic nitrogen compounds and many others. For example, most antimicrobial compounds produced by the legume family plants (Fabaceae) are isoflavones, isoflavanes, related pterocarpans and stilbenes. For the nightshade family (Solanaceae) terpenic compounds are characteristic, for plants of the family of crucifers (Brassicaceae) indole derivatives are typical, and the representative compounds for the carrots family (Apiaceae) are polyacetylenes. This section gives only a short overview of the major antimicrobial compounds occurring

in food raw materials and foods, and aims to demonstrate the chemical diversity of these compounds. Antimicrobial effects are also exhibited by many other compounds described in previous chapters.

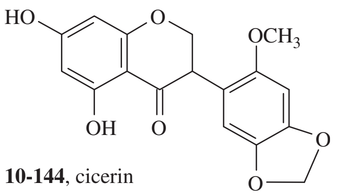
10.3.3.6.1 Isoflavones and isoflavanones

A number of isoflavones exhibit, in addition to oestrogenic activity, antimicrobial activity and other toxic effects. Examples of isoflavones exhibiting antimicrobial properties are prenylated isoflavones of legumes listed in Section 10.3.3.5.1, which occur mainly on the surface of leaves and other aboveground plant parts. Examples of such compounds are wighteon and luteon, which occur in lupines (*Lupinus* spp.) together with other related compounds.

Isoflavanones occur predominantly in legumes as 6′′-malonylglucosides. Examples of isoflavanones are homoferreirin (**10-143**) and cicerin (**10-144**), which occur in chickpea (*Cicer arietinum*).



10-143, homoferreirin



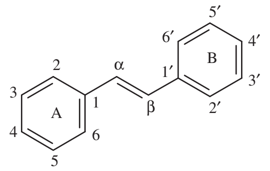
10-144, cicerin

10.3.3.6.2 Pterocarpans

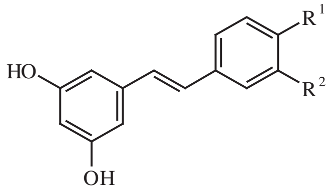
Pterocarpans of legume plants with the properties of phytoalexins are glyceollins in soya beans (*Glycine max*), medicarpin and maackiain in chickpeas (*Cicer arietinum*), pisatin in pea seeds (*Pisum sativum*) and phaseolin in common beans (*Phaseolus vulgaris*). Pterocarpans occur preferentially as 6′′-malonylglucosids. In addition to antimicrobial effects they also exhibit other biological effects (see Section 10.3.3.5.1).

10.3.3.6.3 Stilbenes

Stilbenes (also called diarylethanoids) are a group of substituted plant secondary metabolites with the structure of C_6–C_2–C_6. Stilbenes are hypothetically derived from the hydrocarbon (*E*)-1,2-diphenylethylene, which is known as (*E*)-stilbene or *trans*-stilbene (**10-145**). Ring A usually carries two hydroxy groups in the *m*-position, whereas ring B is replaced by hydroxy and methoxy groups in the *o*-, *m*- or *p*-position. Free stilbenes accompanied by the corresponding glycosides are found in small quantities in several plant species. Their biosynthesis is closely related with biosynthesis of isocoumarins (see Section 10.3.3.5.2). The basic member of a number of common stilbenes is (*E*)-pinosylvin (stilbene-3,5-diol). It occurs along with other stilbenes (**10-146**) especially in conifers, for example in pines (*Pinus* spp., Pinaceae).



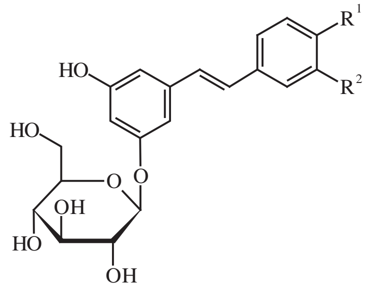
10-145, (*E*)-stilbene



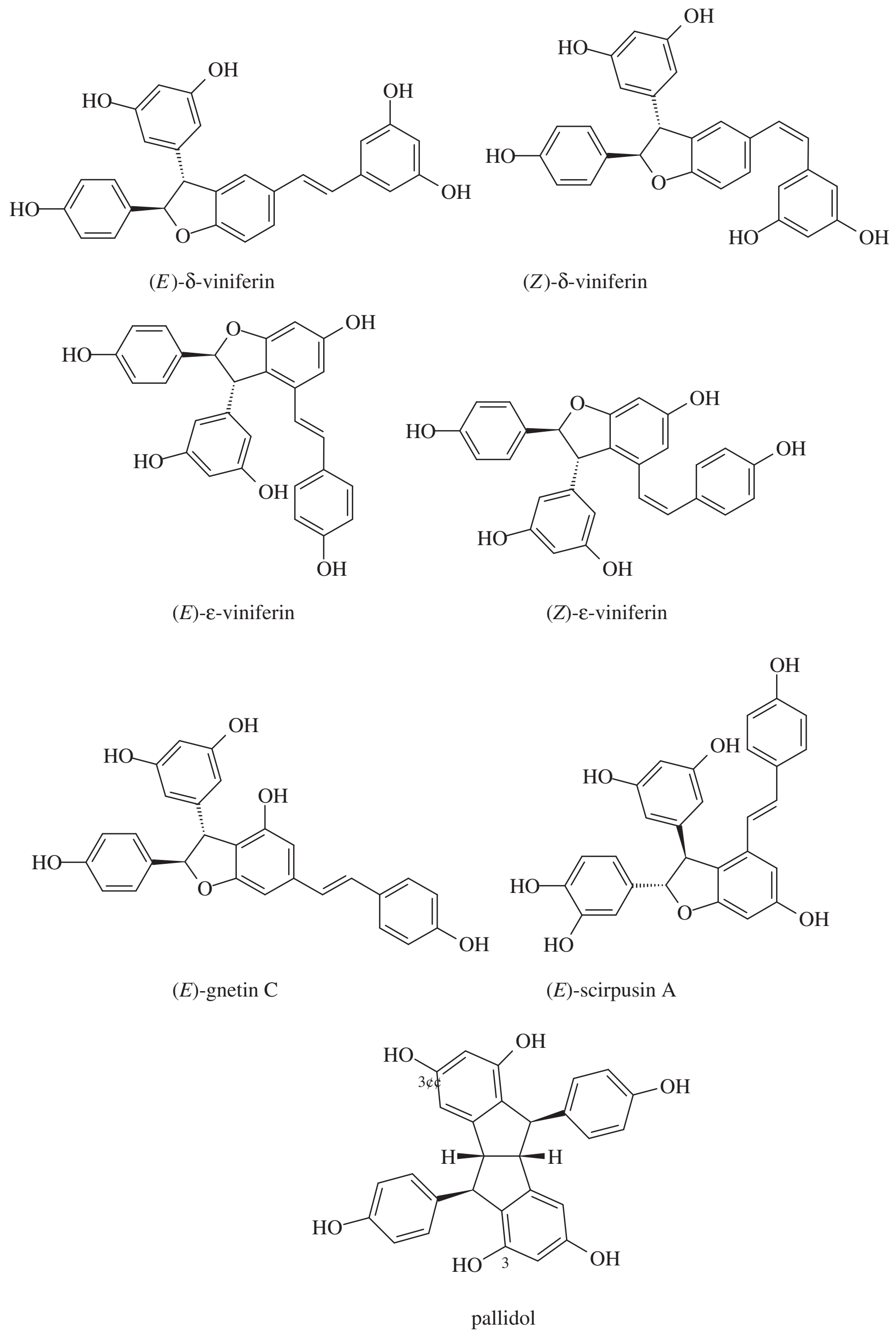
10-146, (*E*)-pinosylvin, $R^1 = R^2 = H$
(*E*)-resveratrol, $R^1 = OH, R^2 = H$
(*E*)-piceatannol, $R^1 = R^2 = OH$

The representative of stilbenes with antimicrobial and antioxidant effects is (*E*)-resveratrol (3,4′,5-trihydroxystilbene, **10-146**), an antifungal substance produced by a relatively limited number of plant species in response to biotic and abiotic stress and UV light irradiation. Resveratrol is synthesised by common grape vine (*Vitis vinifera*, Vitaceae), some legumes, such as peanuts (*Arachis hypogea*, Fabaceae), conifers and some other plants.

Resveratrol in grapes is mainly present in the skins of red grape varieties. From here it passes into the wine in amounts of about 1–5 mg/l. It is accompanied by (*Z*)-resveratrol and some oxidation products, such as (*E*)- and (*Z*)-piceatannol, (*E*)- and (*Z*)-3,3′,4′,5,-tetrahydroxystilbene. Larger amounts of resveratrol and piceatannol are bound in the form of β-D-glucopyranosides. The glucopyranoside of (*E*)-resveratrol is called (*E*)-piceid and the glucopyranoside of (*E*)-piceatannol is known as (*E*)-astringin (**10-147**). Resveratrol, its dimer ε-viniferin, dehydrodimer δ-viniferin, also known as viniferifuran, analogues of some other dimers (**10-148**), trimeric products, such as α-viniferin (**10-149**), resveratrol tetramer β-viniferin, tetramer hopeaphenol and a more



10-147, (*E*)-piceid, $R^1 = OH, R^2 = H$
(*E*)-astringin, $R^1 = R^2 = OH$

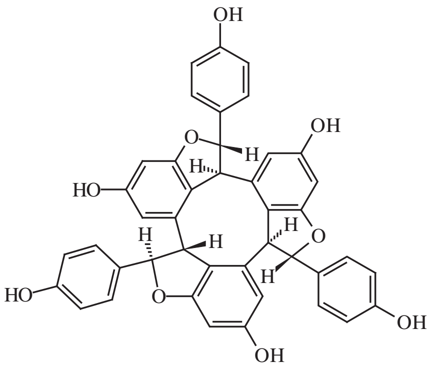


10-148, resveratrol dimers and analogues

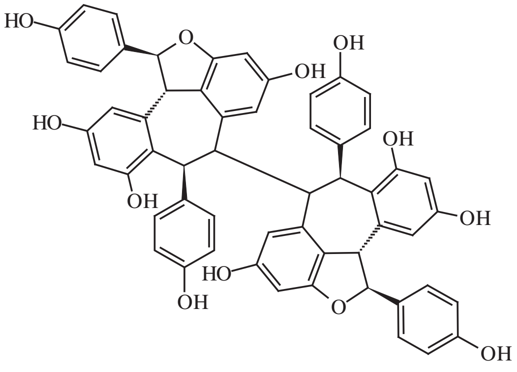
highly polymerised oligomer of resveratrol referred to as γ-viniferin are found in wine grapes. They show even higher antioxidant and fungicidal activity than resveratrol. In recent years the anticarcinogenic and cardioprotective effects of resveratrol have been intensively studied. The plants of the genus *Vitis* (Vitaceae) contain many other stilbenoids that are of chemotaxonomic interest.

The stilbenes that have been reported for several varieties from peanuts (*Arachis hypogaea*), in leaves, roots and seeds, seem to be derived mainly from (*E*)-resveratrol (**10-146**). Examples of peanut stilbenes are (*E*)-piceatannol (**10-146**), (*E*)-piceid (**10-147**), prenylated stilbenes arachidins, (*E*)-4′-deoxyarachidin-2 (chiricanine A), (*E*)-4′-deoxyarachidin-3 (arahypin-1), (*E*)-3′-(2″,3″-dihydroxy-3″-methylbutyl)resveratrol (arahypin-2), (*E*)-4-(2″,3″-dihydroxy-3″-methylbutyl)resveratrol (arahypin-3), (*E*)-4-(2″,3″-dihydroxy-3″-methylbutyl)-4′-deoxyresveratrol (arahypin-4) and arahypin-5 (**10-150**).

Hydroxysubstituted stilbenes are often methylated. Examples of such compounds are (*E*)-pallidol (**10-148**), which occurs in Riesling wine as 3-*O*-glucopyranoside and 3,3″-di-*O*-glucopyranoside. Another example is 3,3′,5-trihydroxy-4′-methoxystilbene, which

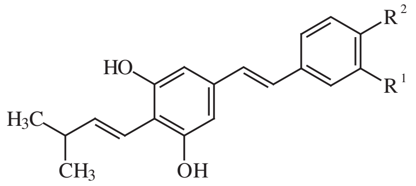


α-viniferin

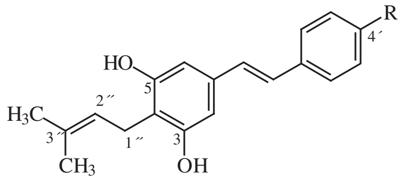


hopeaphenol

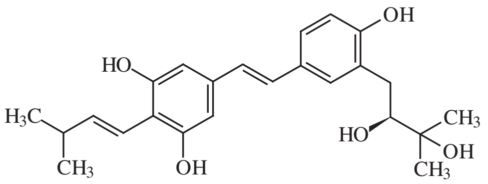
10-149, resveratrol trimers and analogous tetramers



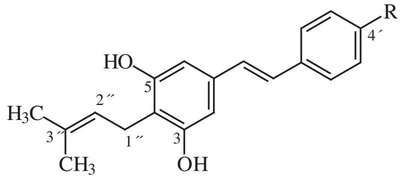
arahypin-1, $R^1 = R^2 = H$
arachidin-3, $R^1 = H$, $R^2 = OH$
arachidin-1, $R^1 = R^2 = OH$



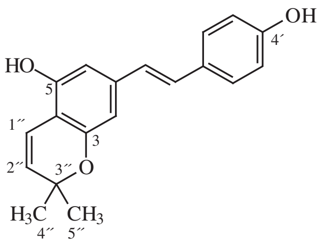
chiricanine A, R = H
arachidin-2, R = OH



arahypin-2



arahypin-4, R = H
arahypin-3, R = OH



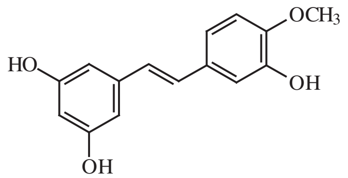
arahypin-5

10-150, arachidins, arahypins and related compounds

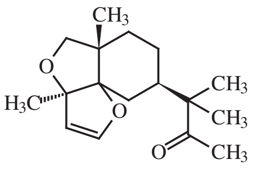
is called rhapontigenin. It occurs as 3-*O*-β-ᴅ-glucopyranoside called rhapontigenin (**10-151**), for example, in rhubarb (*Rheum undulatum*, Polygonaceae), which is used for its haemostatic effects in Korea and Japan. It also displays antioxidant and antimicrobial properties.

10.3.3.6.4 Terpenoids

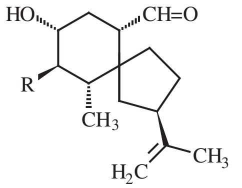
Terpenoid phytoalexins are present in economically important solanaceous plants (Solanaceae). Phytuberin (**10-152**), lubimin and 4-hydroxylubimin (**10-153**), along with steroid glycosides, are important representatives of sesquiterpenic phytoalexins of potatoes (*Solanum tuberosum*). Lubimin is also a major phytoalexin of eggplants (*S. melongena*). Another terpenoid is rishitin (**10-154**) occurring in potatoes and tomatoes (*S. lycopersicum*). Capsidiol (**10-155**), a phytoalexin of bell peppers (*Capsicum* spp.) and tobacco (*Nicotiana* spp.) also shows antimicrobial effects, as do the active substances of hot peppers known as capsaicinoids (see Section 10.3.3.1.7), and tobacco alkaloids (see Section 10.3.3.1.1). Several



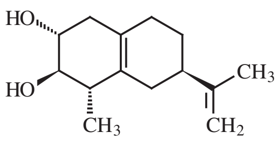
10-151, rhapontigenin



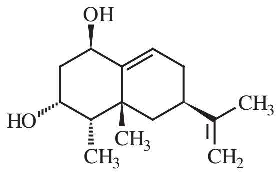
10-152, phytuberin



10-153, lubimin, R = H
4-hydroxylubimin, R = OH

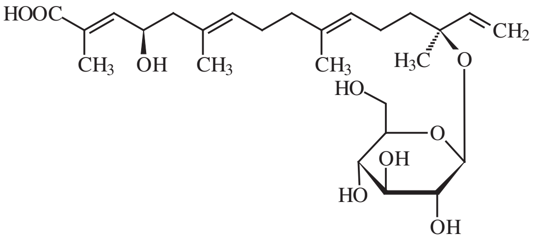


10-154, rishitin



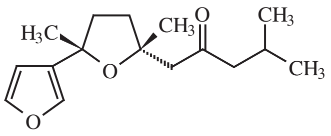
10-155, capsidiol

diterpenoid glycosides, named capsianosides from bell peppers exhibit antihypertensive effects and have been found to be related to the improvement and prevention of hypertension. An example of these diterpenoid glycosides is capsianoside I (**10-156**).

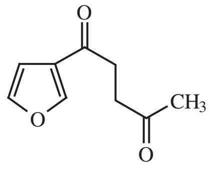


10-156, capsianoside I

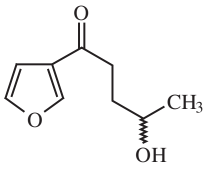
Structurally related to terpenoids are the so-called furanoterpenoids. A number of these derivatives can be found, for example, in sweet potatoes (*Ipomoea batatas*, Convolvulaceae). The first phytoalexin isolated from the crop affected by the mould *Fusarium solani* was hepatotoxic ipomeamarone (**10-157**). Also of toxicological concern are four other related derivatives: ipomeanine (**10-158**), a mixture of enantiomers of ipomean-4-ol (**10-159**) and ipomean-1-ol (**10-160**) and a mixture of diastereomers of ipomea-1,4-diol (**10-161**). The pneumotoxin (a respiratory tract toxicant) ipomean-4-ol is mainly responsible for the toxic effects of sweet potatoes. The use of mechanically damaged and microbially infected sweet potato as feed for cattle represents a significant risk, as in some instances this has led to the death of the livestock due to pulmonary oedema that were incurred. It seems very likely that these substances significantly contribute to the increased incidence of chronic respiratory diseases in the Pacific regions, where sweet potatoes are an important food.



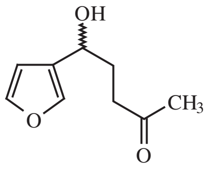
10-157, ipomeamarone



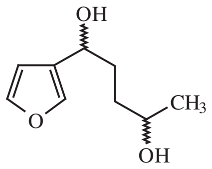
10-158, ipomeanine



10-159, (±)-ipomean-4-ol



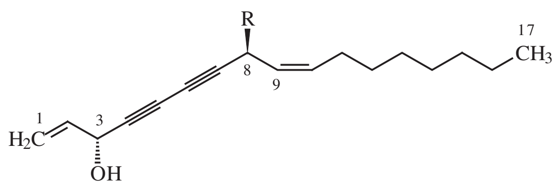
10-160, (±)-ipomean-1-ol



10-161, ipomean-1,4-diol

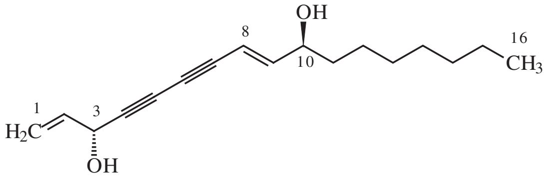
10.3.3.6.5 Polyacetylenes

An example of bitter phytoalexins of this group, found in carrots (*Daucus carota*, Apiaceae), parsley, parsnip, fennel, celery, celeriac and other umbelliferous vegetables, and also in ginseng (*Panax ginseng*, Araliaceae) and ivies (*Hedera* spp., Araliaceae) is (3*R*,9*Z*)-1,9-heptadecadiene-4,6-diyn-3-ol, known as falcarinol or carotatoxin or panaxynol (**10-162**). In carrots, falcarinol may occur in a concentration of about 290 mg/kg dry matter. The corresponding 3-ketone is a constituent of ginseng. Another polyacetylene of carrots is a product of falcarinol oxidation, which is (3*R*,8*S*,9*Z*)-heptadeca-1,9-dien-4,6-diyne-3,8-diol, known as falcarindiol (**10-162**). This diol occurs in about the same concentrations as falcarinol (240 mg/kg dry matter) and is accompanied by the corresponding 3-acetate. Falcarinol and falcarindiol were also found in parsnip in concentrations of 1600 and 5770 mg/kg dry matter, respectively, and in fennel (40 and 240 mg/kg dry matter,



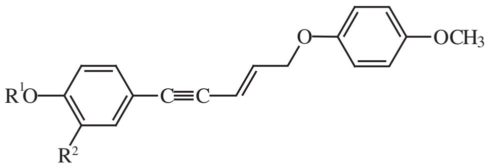
10-162, falcarinol, R = H
falcarindiol, R = OH
8-*O*-methylfalcarindiol, R = OCH_3

respectively). Falcarindiol (2320 mg/kg dry matter), (3*R*,8*S*,9*Z*)-8-*O*-methylfalcarinol (**10-162**) and (3*R*,8*E*,10*S*)-panaxydiol (**10-163**) occur in parsley at concentrations of 2320, 350 and 120 mg/kg, respectively. Celeriac contains up to 1620 mg/kg dry matter falcarinol, 2070 mg/kg dry matter falcarindiol, 170 mg/kg dry matter 8-*O*-methylfalcarinol and 60 mg/kg dry matter falcarindiol. The recognition threshold concentrations of falcarinol, falcarindiol and its acetate are 20, 40 and 60 mg/l.



10-163, panaxydiol

Asparenyn, 1-methoxy-4-[5-(4-methoxyphenoxy)pent-3-en-1-ynyl]benzene, 2-hydroxyasparenyn and asparenyol are examples of acetylenic compounds with cancer chemopreventive properties, which occur in asparagus (*Asparagus officinalis*, Asparagaceae) (**10-164**).

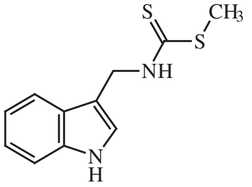


10-164, asparenyol, $R^1 = R^2 = H$
asparenyn, $R^1 = CH_3$, $R^2 = H$
2-hydroxyasparenyn, $R^1 = CH_3$, $R^2 = OH$

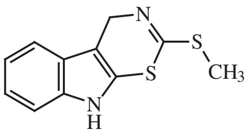
Except for antibacterial and antifungal activities, aliphatic polyacetylenes of the falcarinol type have other interesting bioactivities, including anti-inflammatory, anti-platelet-aggregatory, neuritogenic, serotonergic and potential anticancer effects. A medicinal usage of pure polyacetylenes is not feasible, because of their pronounced chemical instability and their ability to induce allergic reactions. However, consumption of food containing polyacetylenes might have a chemopreventive benefit. Adverse effects due to an excessive intake of polyacetylenes with the human diet are not to be expected, because polyacetylenes have a bitter off-taste in higher concentrations and are one of the main group of compounds contributing to the bitter taste of stored carrots.

10.3.3.6.6 Indoles

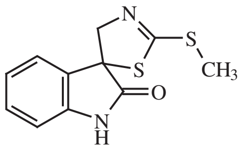
Interest in phytoalexins of the family Brassicaceae, called brassinins, began in the late 1980s when the first phytoalexin was identified in Chinese cabbage (*Brassica chinensis*). About 20 phytoalexins characteristic for this plant family are known today. Their common feature is the indole ring substituted at C-3 or C-2 by substituents containing nitrogen and sulfur. The structures of these compounds are illustrated in the structures of four commonly occurring indole phytoalexins: brassinin (**10-165**), cyclobrassinin (**10-166**), spirobrassinin (**10-167**) and brassilexin (**10-168**). Phytoalexins of cruciferous vegetables include also glucosinolates (see Section 10.2.3.3).



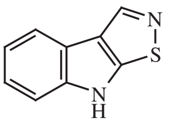
10-165, brassinin



10-166, cyclobrassinin



10-167, spirobrassinin



10-168, brassilexin

Biological effects

Biological activity of phytoanticipins and phytoalexins (and natural pesticides in general) is not limited to microorganisms and other pests, but affects virtually all organisms. These materials exhibit a wide spectrum of effects, from the relatively harmless, such as the hallucinogenic and narcotic effects of myristicin (see Section 8.2.13.2), to the toxic effects of carcinogenic furanocoumarins (see Section 10.3.3.5.2). Mechanisms of biological effects of most phytoanticipins and phytoalexins are not known, and practical toxicological data on the effect of long-term exposure (chronic toxicity) do not exist.

10.3.3.7 Other natural antioxidants

The use of antioxidants dates back to ancient times, when herbs and spices were used in food preservation. A number of plant food materials have antioxidant properties. Particularly effective plants are rosemary and sage, but oregano, thyme, cloves, turmeric, oatmeal and other materials are also effective. Natural antioxidants (mainly derived from plant extracts) often have limited use, because they may resemble the odour of the plant or may have a bitter taste. An overview of natural antioxidants occurring in significant food materials is given in Table 10.20. The issue of natural and synthetic antioxidants approved in the EU as food additives is addressed in Section 11.2.2.

10.3.3.7.1 Simple phenols

Antioxidant and antimicrobial effects are found in some simple phenols (especially hydroquinone, guaiacol, isoeugenol and salicylaldehyde) that occur as components of smoke, which has been used for smoking foods since ancient times. Phenols, which are components of some common spices, also have high antioxidant activities. In common thyme (*Thymus vulgaris*, Lamiaceae), for example, thymol and carvacrol occur (see Section 8.2.8.1).

Table 10.20 Natural antioxidants of important food commodities.

Material	Compounds with antioxidative activities
Fruits	
Olives (*Olea europea*)	Phenolic acids (*p*-coumaric and others), their glycosides (verbascoside), phenols (tyrosol, hydroxytyrosol), oleuropein
Spices	
Rosemary (*Rosmarinum officinalis*)	Diterpenes (carnosic acid, carnosol and others), phenolic acid esters (rosmarinic acid)
Sage (*Salvia officinalis*)	Diterpenes (carnosic acid, carnosol and others)
Tyme (*Thymus vulgaris*)	Simple phenols (thymol, carvacrol) and their dimers (diterpenes)
Turmeric (*Curcuma longa*)	Curcuminoids (diarylheptanoids and their lower homologues)
Ginger (*Zingiber officinale*)	Gingerols, shogaols, zingerone, curcuminoidsy
Vegetables	
Onion (*Allium cepa*)	Flavonoids (quercetin), phenolic acids
Peppers (paprika) (*Capsicum annuum, C. frutescens*)	Capsaicinoids and related compounds
Cereals and oilseeds	
Wheat (*Triticum aestivum*)	Phenolic acids (esters, glycosides), tocopherols, flavonoids, phospholipids
Rye (*Secale cereale*)	Phenolic acids (esters, glycosides), tocopherols, flavonoids, phospholipids
Barley (*Hordeum vulgare*)	Tyrosine, tyramine, phenolic acids (esters, glycosides), tocopherols, flavonoids, phospholipids, lignans
Oats (*Avena sativa*)	Avenanthramides, phenolic acids (esters, glycosides), tocopherols, flavonoids, phospholipids
Rice (*Oryza sativa*)	Flavones (isovitexin), phenolic acids (esters, glycosides), tocopherols
Soya (*Glycine max*)	Tocopherols, isoflavones and their glycosides, phenolic acids, phospholipids
Sesame seed (*Sesamum indicum*)	Lignans and their degradation products
Peanuts (*Arachis hypogaea*)	Phenolic acids (*p*-coumaric and others), flavonoids (taxifolin, in hulls eriodictyol and luteolin), tanns, tocopherols
Rapeseed (*Brassica napus*, *B. rapa*)	Phenolic acids (their esters and glycosides), mainly (*E*)-sinapic acid and sinapine, tannins, tocopherols

10.3.3.7.2 Phenolic acids and their derivatives

Phenolic acids, primarily acids with structures C_6–C_1 (benzoic acid and its derivatives) and C_6–C_3 (cinnamic acid and its derivatives) are normal constituents of plant materials (see Section 8.2.6.1.6). Some aromatic carboxylic acids, such as 2-hydroxybenzoic (salicylic) and 4-hydroxybenzoic acids, are components of smoke used for smoking foods.

Phenolic acids and their derivatives exhibit the effects of primary antioxidants. Their activity depends on the number of hydroxyl groups in the molecule. Generally cinnamic acids and *o*-diphenols are more active as antioxidants (e.g. caffeic acid

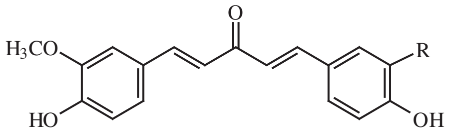
and its ester chlorogenic acid or ester of caffeic acid and 3,4-dihydroxyphenyllactic acid known as rosmarinic acid, see Section 8.2.7.1.1). In addition to these esters (depsides), many other phenolic acid derivatives, such as amides and glycosides, show antioxidant activity.

10.3.3.7.3 Lignans

Simple diarylbutanoids as well as complex bisepoxylignans (see Section 10.3.3.5.1) and other lignans have antioxidant activity and other biological effects. Some lignans are also active as phytooestrogens and anticancerogenic agents.

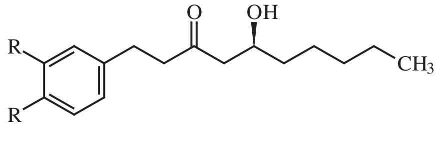
10.3.3.7.4 Curcuminoids and related compounds

Yellow pigments of turmeric (*Curcuma longa*, Zingiberaceae), which are classified as curcuminoids and diarylheptanoids (see Section 9.6), have antioxidant effects. Also potent antioxidants are curcuminoids occurring in other plants of the genus *Curcuma*, such as *C. xanthorrhiza*, originating from Indonesia, which contains the lower homologues of yellow curcuminoids belonging to diarylpentanoids (**10-169**), where R = H or OCH_3.

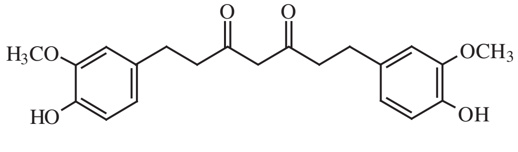


10-169, turmeric diarylpentanoids

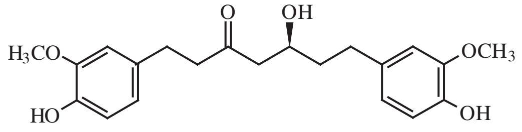
Ginger rhizome (*Zingiber officinale*) contains hot gingerols, shogaols and zingerone (see Section 8.3.7.3) that are highly active antioxidants. In addition to these compounds, several tens of compounds occur in various ginger species, which are structurally related to gingerols, shogaols or curcuminoids. Examples are methoxy-6-gingerol, deoxy-6-gingerol (**10-170**), tetrahydrocurcumin (**10-171**) and hexahydrocurcumin (**10-172**). Ginger rhizome also contains more complex compounds, examples of which are the so-called phenylbutenoids cassumunins A and B (**10-173**). A related species of ginger (*Z. montanum*, syn. *Z. cassumunar*) contains, in addition to curcumin, cassumunins A, B and C (**10-174**), together with kassumunarins A (**10-175**), B and C (**10-176**).



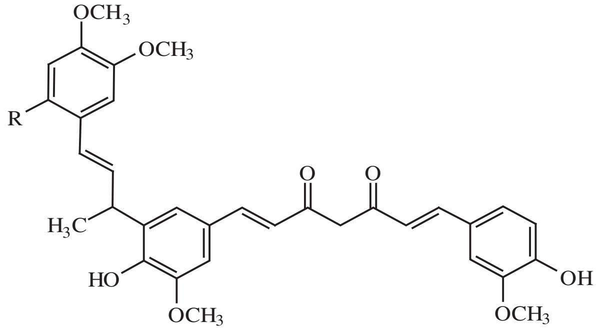
10-170, methoxy-6-gingerol, $R^1 = OCH_3$, $R^2 = OCH_3$
deoxy-6-gingerol, $R^1 = H$, $R^2 = OH$



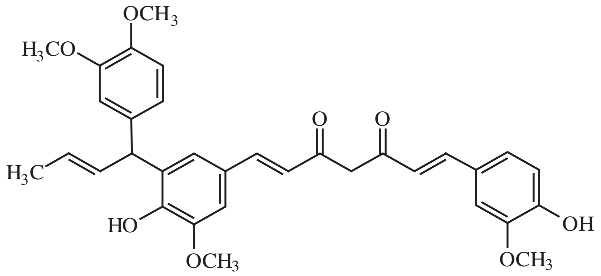
10-171, tetrahydrocurcumin



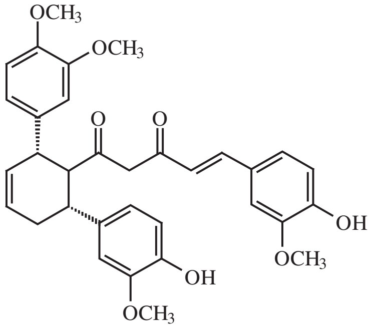
10-172, hexahydrocurcumin



10-173, cassumunin A, R = H
cassumunin B, $R = OCH_3$



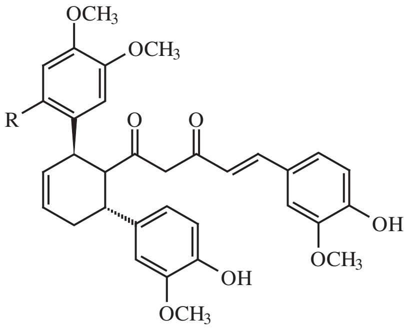
10-174, cassumunin C



10-175, cassumunarin A

An unusual diarylheptanoid (4*E*,6*E*)-1,7-bis(4-hydroxyphenyl) hepta-4,6-dien-3-one (**10-177**) was isolated from fruits of a banana (Musa x paradisiaca, cultivar derived from *M. acuminata* and *M. balbisiana*) together with a bicyclic diarylheptanoid, *rel*-(3*S*,4a*R*,10b*R*)-8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4a,5,

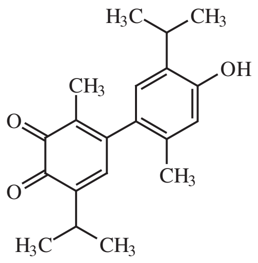
6,10b-tetrahydro-3*H*-naphtho[2,1-*b*]pyran, as well as 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene, hydroxyanigorufone with chemopreventive activities and cytotoxic 2-(4-hydroxyphenyl)naphthalic anhydride.



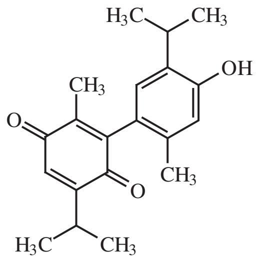
10-176, cassumunarin B, R = H
cassumunarin C, R = OCH_3

10.3.3.7.5 Terpenoids

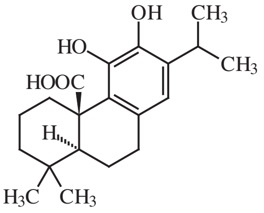
Mint plants (Lamiaceae), for example in common thyme *(Thymus vulgaris*), contain substituted biphenyls derived from monoterpenic alcohol thymol, their *o*-quinones (**10-178**) or *p*-quinones (**10-179**), which exhibit marked antioxidant activities. Among the most active natural antioxidants with anti-inflammatory effects is carnosic acid, also known as rosmaricine (**10-180**), which is accompanied by carnosol (picrosalvin, **10-181**). These two antioxidants represent about 15% by weight of commercial extracts of rosemary (*Rosmarinus officinalis*) and more than 90% of their antioxidative activity. The carnosic acid content in fresh spices is about 1–2%.



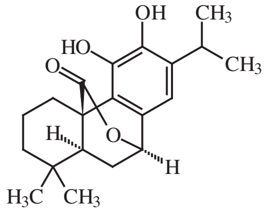
10-178, thyme *o*-quinone



10-179, thyme *p*-quinone

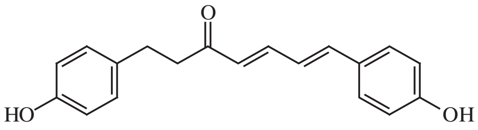


10-180, carnosic acid

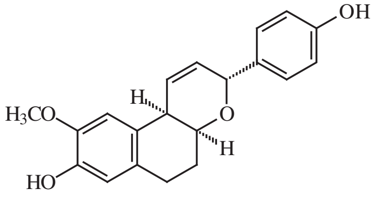


10-181, carnosol

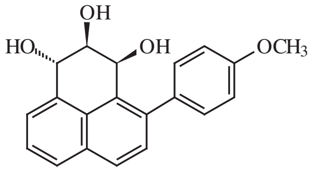
Carnosic acid is unstable and is transformed into carnosol. Other active products are rosmanol (7α-isomer, **10-182**), epirosmanol (7β-isomer) and 7-methylepirosmanol (**10-183**). Other minority compounds of rosemary extracts are isorosmanol (**10-184**), rosmariquinone (**10-185**) and rosmaridiphenol (**10-186**), which



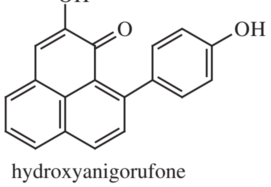
1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one



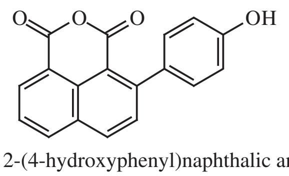
8-hydroxy-3-(4-hydroxyphenyl)-9-methoxy-4a,5,6,10b-tetrahydro-3*H*-naphtho[2,1-*b*]pyran



1,2-dihydro-1,2,3-trihydroxy-9-(4- methoxyphenyl)phenalene



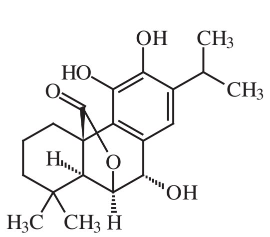
hydroxyanigorufone



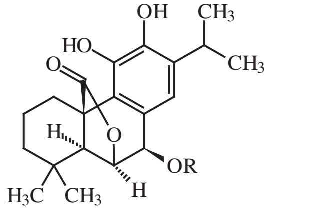
2-(4-hydroxyphenyl)naphthalic anhydride

10-177, banana diarylheptanoids

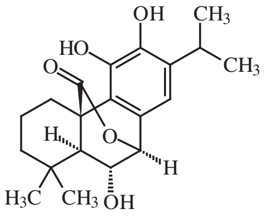
is related to tropones. The same compounds are also found in common (garden) sage (*Salvia officinalis*).



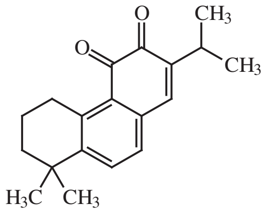
10-182, rosmanol



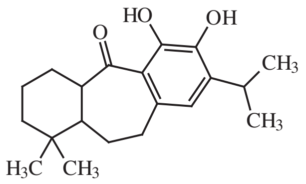
10-183, epirosmanol, R = H
7-methylepirosmanol, R = CH_3



10-184, isorosmanol



10-185, rosmariquinone



10-186, rosmaridiphenol

Extract of rosemary is currently approved in the EU as a natural antioxidant (E392). Carnosic acid has a typical *o*-diphenol structure and is easily oxidised. The antioxidation mechanism is based on a coupling reaction with the peroxyl radical at the 12- or 14-position of carnosic acid and subsequent transformation reactions of intermediates to an *o*-quinone and a hydroxy *p*-quinone (Figure 10.13).

An important group of substances derived from diterpenoids are quinones of red (Chinese) sage (*Salvia miltiorrhiza*). Antioxidative properties are related to their reduced glycosidic forms. The drug is used as a traditional medicine in China and Southeast Asia countries. The active substances can actually be considered as derivatives of phenanthrenequinones, but their biochemical origin is different.

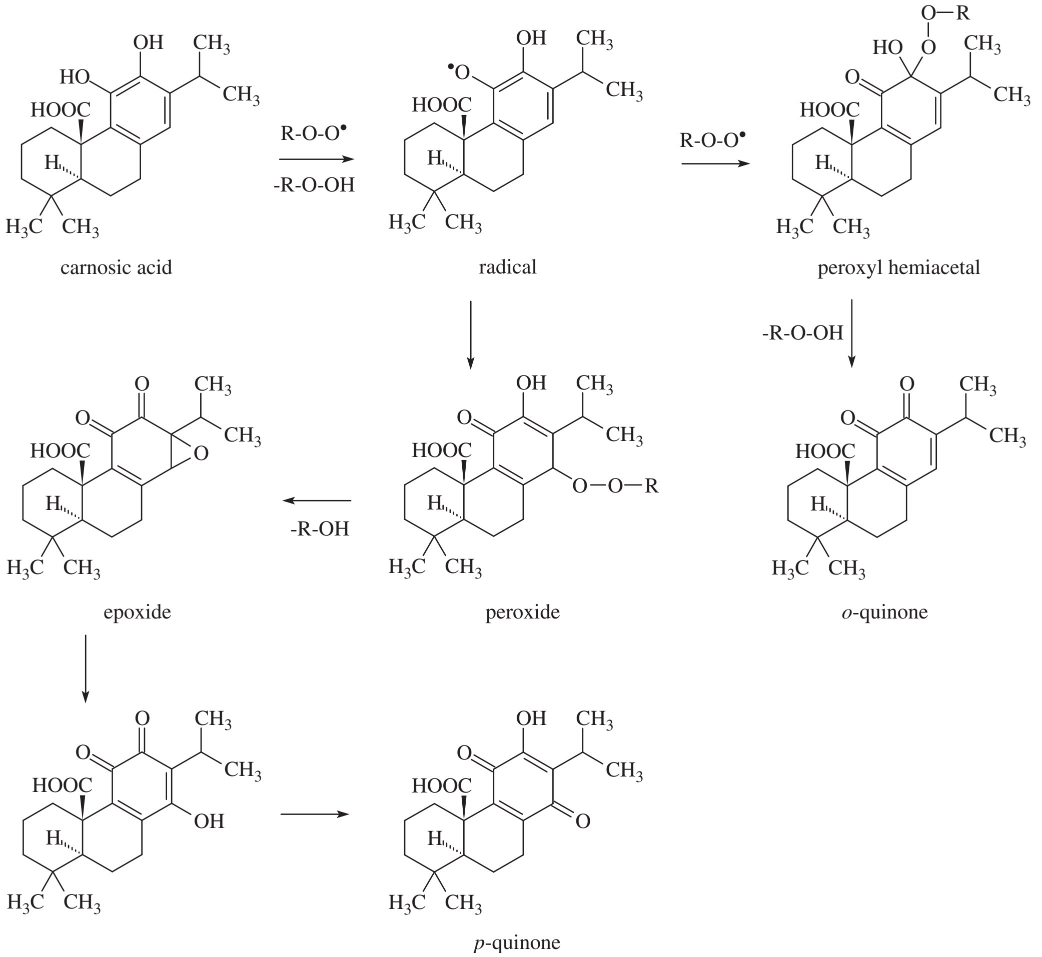
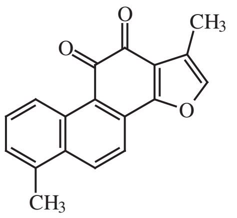
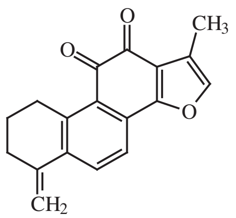


Figure 10.13 Antioxidant mechanism of carnosic acid.

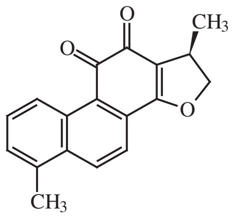
Some furanophenathrenequinones are exemplified in formulae **10-187** to **10-189**. In addition to these compounds, another group of quinones includes their isopropyl derivatives, such as rosmariquinone (**10-185**), which is also found in rosemary, miltirone (**10-190**), dehydrorosmariquinone (**10-191**), danshenxinkuns A (10-190), B and C (**10-192**). In addition to antioxidant activity, these substances also have sedative, antimicrobial, anticoagulant, anti-inflammatory and other effects. In addition to these compounds, other components include rosmarinic acid (**8-83**) and a number of its derivatives.



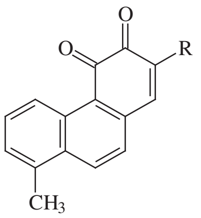
10-187, tanshinone I



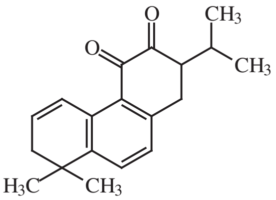
10-188, methylenetanshinone



10-189, (*R*)-dihydrotanshinone

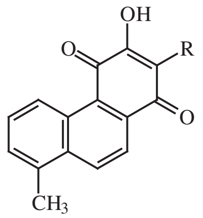


10-190, miltirone I, R = $CH(CH_3)_2$
danshenxinkun A, R = $CH(CH_3)CH_2OH$



10-191, dehydrorosmariquinone

The antioxidant potential of plant materials, such as rosemary, also comes from some triterpenic acids such as betulinic, oleanolic and ursolic acids, triterpenic alcohols (β-amyrin and others, see Section 3.7.4.1.1) and derived saponins (see Section 10.3.2.2). Usually low antioxidant activity (up to temperatures around 180 °C) is



10-192, danshenxinkun B, R = $CH(CH_3)_2$
danshenxinkun C, R = CH_3

exhibited by phytosterols, the most active compound of which is Δ^5-avenasterol (see Section 3.7.4.1.3).

10.3.3.7.6 Flavonoids

Flavonoid substances are primary antioxidants. Flavonols and 5-hydroxysubstituted flavones also bind metals into inactive complexes. Important for the antioxidant activity of flavonoids is the number of hydroxyl groups in the molecule and their positions. Active compounds are all dihydroxy derivatives with hydroxyl groups at positions C-3′ and C-4′. The presence of other hydroxy groups in ring B further increases the antioxidant activity (e.g. robinetin and myricetin with an additional hydroxyl group at C-5′ are more active as antioxidants than quercetin and fisetin). Low antioxidative activity is exhibited by flavonoids with one hydroxy group in ring B (such as flavanones naringenin and hesperetin). Other important functional groups include the carbonyl group at C-4 and free hydroxyl group at C-3 (or C-5). Very efficient antioxidants have two hydroxyl groups in the *o*-position in one ring and two hydroxy groups in the *p*-position in other rings, such as 3,5,8,3′,4′- and 3,7,8,2′,5′-pentahydroxy derivatives.

10.3.3.7.7 Other antioxidants

In biological materials, some vitamins (especially vitamins C and E) and a number of nitrogen and sulfur compounds act *in vivo* as substances with potential antioxidant carotenoids and related polyenes. For example, nitrogen compounds with significant antioxidant activities include certain alkaloids, uric acid and other purines, some peptides, amino acids, biogenic amines and tetrapyrrole pigments. Important sulfur compounds with antioxidant activity are sulfur amino acids (cysteine and methionine), derived peptides (glutathione) and some proteins. An antioxidant of animal and plant tissues also found in microorganisms is lipoic acid and derived sulfides and polysulfides. Some fungi contain as an antioxidant a derivative of histidine known as ergothioneine (**2-16**). In marine animals there is a mercaptohistidine derivative ovothiol A (**2-55**). Dipeptide carnosine also exhibits antioxidant activity in animal tissues.

Biological effects

The evaluation of natural antioxidants is highly problematic, since many of them have different biological activities. Some flavonoids, for example, exhibit spasmolytic, anti-inflammatory and

anticancerogenic effects, some also act as phytoalexins protecting plants against viral and microbial infections, while certain compounds have oestrogenic, carcinogenic and other effects.

10.3.3.8 Lectins

One of the protection mechanisms of plants lies in the accumulation of certain proteins in seeds and vegetative parts related to reproduction. These organs of plants are unacceptable, unpalatable or toxic to parasites and predators. Such proteins are inhibitors of proteases (see Section 10.2.1.1), inhibitors of saccharases (see Section 10.2.1.2) and lectins.

10.3.3.8.1 Structure, nomenclature and occurrence

Lectins, formerly called phytohaemagglutinins, were originally defined as proteins capable of red blood cells (erythrocytes) agglutination in animal organisms. Their activity is due to their interaction with sugars, which are components of glycoproteins or glycolipids in cell membranes. The current definition refers to lectins, as all proteins with at least one centre other than the active (catalytic) centre by which proteins can reversibly bind to specific monosaccharides and oligosaccharides. Many lectins can bind to monosaccharides and their derivatives (such as D-glucose, D-mannose, D-galactose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine and L-fucose), however they have a higher affinity to oligosaccharides that do not occur in plants, but are typical components of animal glycoproteins. Such compounds are, for example, *N*-acetylneuraminic acid and *N*-acetyl-D-galactosamine located as building units in the chains of complex carbohydrates.

Lectins are a highly heterogeneous group comprising several hundreds of plant proteins, which are divided into three groups:

- **merolectins**
- **hololectins**
- **chimerolectins**.

Merolectins are simple proteins unable to agglutinate cells, which contain only one centre binding sugars and no catalytic centre. Merolectin is, for example, the protein of amaranth (*Amaranthus caudatus*, Amaranthaceae), which binds polysaccharide chitin.

Hololectins are proteins containing at least two centres binding sugars and, like merolectins, have no catalytic functions. They behave as true agglutinins. The majority of plant lectins are hololectins. An example is soya bean lectin (SBA, abbreviation of Soya Bean Agglutinin), a tetrameric glycoprotein (or metalloprotein as it contains one binding site for Mn^{2+} and four sites for binding transition metals), with a relative molecular weight of 120 kDa. It is composed of two structurally slightly different subunits (30 kDa) and contains four centres binding *N*-acetyl-D-galactosamine.

Chimerolectins are complex proteins containing one to two centres binding sugars and an independent centre with catalytic or other biological activity. Depending on the number of sugar binding centres, chimerolectins behave either as merolectins or as hololectins. For example, lectin inactivating ribosomes type 2, such as toxic protein ricin occurring in castor seeds (*Ricinus communis*, Euphorbiaceae), has a catalytically active A chain covalently linked to two chains B by disulfide bridges. Each of these two polypeptide chains B contains one centre, which binds sugars; therefore ricin behaves as a hololectin.

The majority of known lectins belong to one of four major subgroups:

- legume lectins
- mannose-binding lectins (monocot plant lectins)
- chitin-binding lectins
- ribosome type 2-inactivating lectins.

The most important mannose-binding lectins occur in different families of plants, mainly Amarillidaceae, Araceae, Liliaceae and Orchidaceae. Chitin-binding lectins are located in five mutually taxonomically unrelated families of plants, Poaceae, Solanaceae, Urticaceae, Papaveraceae and Amaranthaceae. Some lectins cannot be classified into any of these subgroups, because they appear to belong to other, not yet specified, subgroups.

Lectins are widely distributed in both the plant and animal kingdoms, and are even present in some microorganisms. The maximum amounts are found in the seeds of plants. The most frequently occurring lectins, their content and important properties are shown in Table 10.21. Some lectins traditionally have trivial names, for example the lectin (albumin) of castor seeds is ricin, and the lectin of jack beans (*Canavalia ensiformis*, Fabaceae) is trivially called concavalin A.

10.3.3.8.2 Reactions and changes

Lectins can be denatured by heat and a decrease of their biological activity also occurs during proteolysis. Detoxification procedures of lectins in food materials commonly include soaking and cooking. The efficiency of these operations depends on the time of soaking, temperature and the time of heat treatment. Soaking itself is not sufficient. For example, detoxification of beans by soaking for 16 hours at 22–25 °C decreases the activity of lectins by only 4–5%. Cooking itself is also not appropriate for food detoxification. For example, detoxification of beans by cooking takes about 90 min, while the same material soaked for 16 hours is detoxified during 4–10 min of cooking. Lectins are largely dissolved into the soaking water and the cooking water. It is not recommended to consume this water. Autoclaving, especially at higher pressures, is also a suitable detoxification procedure, but during extrusion, only small losses of lectin activity occur (12–18%).

Cereal products containing soya beans generally show higher residual activity of lectins than other materials. Seed germination leads to a significant decrease in the activity of lectins. The germination time required to substantially reduce the activity of lectins is 4–6 days. Detoxification of common products, such as potatoes, can be done by common manufacturing and culinary practices.

Table 10.21 Main lectins in economically important plants.

Plants	Latin name	Occurrence	Content (g/kg)	Thermal stability[a]	Toxicity in foods	
					Raw	Processed
Legume lectins						
Peanuts	*Arachis hypogaea*	Seeds	0.2–2	Unstable	Yes	Yes
Peas	*Pisum sativum*	Seeds	0.2–2	Unstable	Possibly	No
Common beans	*Phaseolus vulgaris*	Seeds	1–10	Medium	Yes	Possibly
Jack beans	*Phaseolus lunatus*	Seeds	1–10	Medium	Yes	Possibly
Tepary beans	*Phaseolus acutifolius*	Seeds	1–10	Medium	Yes	Possibly
Runner bean	*Phaseolus coccineus*	Seeds	1–10	Medium	Yes	Possibly
Lens	*Lens culinaris*	Seeds	0.1–1	Unstable	Yes	No
Soya beans	*Glycine max*	Seeds	0.2–2	Low	Yes	No
Broad beans	*Vicia faba*	Seeds	0.1–1	Unstable	Possibly	No
Mannose-binding lectins						
Onion	*Allium cepa*	Bulb	<0.01	Medium	No	No
Garlic	*Allium sativum*	Bulb	0.5–2	Medium	No	No
Leek	*Allium ampeloprasum*	Leaf sheaths	<0.01	Medium	No	No
Chitin-binding lectins						
Wheat	*Triticum aestivum*	Seeds	<0.01	High	Yes	Yes
Wheat	*Triticum aestivum*	Germ	0.1–0.5	High	Yes	Yes
Rye	*Secale cereale*	Seeds	<0.01	High	Yes	Possibly
Barley	*Hordeum vulgare*	Seeds	<0.01	High	Yes	Possibly
Rice	*Oryza sativa*	Seeds	<0.01	High	Yes	Possibly
Potatoes	*Solanum tuberosum*	Tubers	0.01–0.05	High	-	Possibly
Tomatoes	*Lycopersicon esculentum*	Fruits	<0.01	High	Possibly	Possibly
Ribosome type 2- inactivating lectins						
Castor oil plant[b]	*Ricinus communis*	Seeds	1–5	Unstable	-	-
Elderberries	*Sambucus nigra*	Fruit	0.01	Medium	Yes	Possibly
Other plant lectins						
Amaranth	*Amaranthus caudatus*	Seeds	0.1–0.5	Unstable	Possibly	Unknown
Banana	*Musa x paradisiaca*	Fruit	<0.01	Unknown	Unknown	Unknown

[a]Thermal stability: high = lectin does not loose activity by heating at 80 °C, medium = lectin does not loose activity by heating at 70 °C, low = lectin does not loose activity by heating at 60 °C, unstable lectin is denatured at 60 °C.
[b]Highly toxic.

10.3.3.8.3 Biological effects

Lectins applied intravenously are highly toxic and some exhibit toxicity even when applied orally. Acute toxicity is usually low, but long-term exposure to even small amounts of lectins can be harmful. Consuming raw or inadequately cooked beans may cause stomach upset, vomiting and diarrhea. In comparison with the lectins of common beans, soya bean lectins are relatively well digested in the small intestine, but they are still antinutritional or toxic compounds. Approximately 60% of ingested lectins passes through the digestive tract unchanged and bind to the carbohydrate receptors of the small intestine epithelium, which is manifested by reduced viability of epithelial cells and a possible increase of small intestine weight as a result of cell hyperplasia. At concentrations of 0.5–0.6%, the growth of experimental animals is slow; at higher concentrations it leads to weight loss and, in some cases, to the death of affected animals.

Non-toxic lectins are lectins of garlic, onions, leeks, tomatoes and amaranth. Of the common lectins, those of peanuts, lentils, peas, common beans and soya beans are slightly toxic, wheat lectins are moderately toxic, and lectins of some beans (such as Jack beans) are highly toxic, while castor seed lectins are lethal (Table 10.21). Some lectins (such as lectins of garlic) have prebiotic effects and inhibit undesirable intestinal microflora (*Escherichia coli*). Often, lectins are not the only toxic substances of the plant material. For example, lectins of soybean seeds participate in their antinutritional and toxic effects at a level of about 25%, but trypsin inhibitors are about 40% and the rest is covered by saponins and other substances.

10.3.3.9 Toxic amino acids

Common proteinogenic amino acids may show some toxic effects in animals, for example L-tryptophan and L-3,4-dihydroxyphenylalanine (dopa). In plants about 700 aminocarboxylic and iminocarboxylic acids occur (see Section 2.2.1.2), which do not occur in proteins and are classified as secondary metabolites. Many of these compounds are structurally similar to the basic (proteinogenic) amino acids and their toxicity may be manifested in a variety of symptoms. The mechanism of toxicity is usually due to one of the following effects:

- competitive inhibition of enzymes because of resemblance to normal substrates
- interference in the activation and transfer of normal amino acid to transfer RNA
- interference in the assembly of amino acids into protein and interruption of protein synthesis
- incorporation into proteins resulting in the formation of non-functional proteins.

With the exception of the first effect, the other effects are fairly subtle metabolic disorders that can lead to diseases in humans and animals rather than to poisoning. Toxicologically important amino acids that are part of human food or animal feed are usually divided into several groups. They are mainly identified as:

- lathyrogenic amino acids (lathyrogens)
- arginine analogues
- analogues of sulfur and selenium amino acids
- other toxic amino acid.

10.3.3.9.1 Structure, nomenclature and occurrence

Lathyrogens

Lathyrogens are toxic amino acids and their derivatives (peptides, nitriles and other substances) occurring in the seeds of certain vetchlings (*Lathyrus* spp.) and vetches (*Vicia* spp.) belonging to the legume family (Fabaceae). Lathyrism is manifested by deformations of lower limbs (**osteolathyrism**), damage to blood vessels (**angiolathyrism**) and disorders of the nervous system (**neurolathyrism**).

Osteolathyrism appears in livestock fed with seeds of some vetchling species, namely sweet pea (*L. odoratus*), caley pea (*L. hirsutus*), tiny pea (*L. pussilus*) and pink vetchling. (*L. roseus*). Forms of osteolathyrism in poultry fed with sweet pea seeds are sometimes called **odoratism**. Osteolathyrism also occurs in people suffering from chronic neurolathyrism. The toxic substance is β-aminopropionitril (see Section 2.2.1.2.4), occurring as such or as the γ-glutamyl derivative. β-Aminopropionitril arises from 2-cyanoethylisoxazolin-5-one, the precursor of which is 2-(3-amino-3-carboxypropyl)isoxazolin-5-one (see **2-74**). The content of β-aminopropionitril in seeds of sweet pea ranges up to 0.8% of dry matter. In vetches, β-aminopropionitril results probably by decarboxylation of cyanoalanine (see Figure 2.2).

Neurolathyrism accompanies consumption of seeds of some vetchlings and vetches. Neuronal activity is shown by L-2,4-diaminobutanoic (L-α,γ-diaminobutyric) acid (see Section 2.2.1.2.9), which is a lower homologue of L-ornithine. It is present in particular in the seeds of flat pea (*L. sylvestris*) and perennial pea (*L. latifolius*), along with one of the degradation products of 2-(3-amino-3-carboxypropyl)isoxazolin-5-one, but it may arise also from L-3-cyanoalanine. 2,4-Diaminobutanoic acid in seeds occurs in quantities up to 1.5% dry weight and is accompanied by 4-(*N*-oxalyl)-L-2,4-diaminobutanoic acid. The main neurolathyrogen is 3-(*N*-oxalyl)-L-2,3-diaminopropanoic acid, also known as β-(*N*-oxalylamino)-L-alanine, which is produced by the reaction of L-2,3-diaminopropanoic acid with oxalyl-CoA and also from L-3-cyanoalanine. This neurotoxin is found primarily in seeds of grass pea (*L. sativus*), red pea (*L. cicera*), winged vetchling (*L. ochrus*) and crimson pea (*L. clymenum*). Its content in seeds of grass pea ranges from 0.1 to 1.5% and in the seeds of red pea from 0.1 to 0.3%. Diaminopropanoic acid similarly occurs in many rattle pods (*Crotalaria* spp.) and acacias (*Acacia* spp., Fabaceae). At a level of about 5% w/w, diaminopropanoic acid is accompanied by the non-toxic α-isomer, which arises from the β-isomer by intramolecular rearrangement via 2,3-dioxopiperazine-5-carboxylic acid (Figure 10.14). The expected reaction sequences of formation of neurotoxic amino acids and other toxic compounds in vetchlings seeds are illustrated in Figure 10.15 (AdoMet, also known as SAM is *S*-adenosyl-L-methionine).

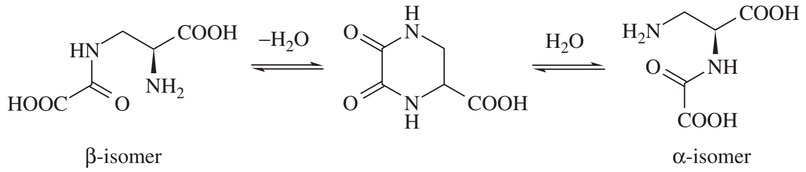


Figure 10.14 Isomerisation of 3-(*N*-oxalyl)-2,3-diaminopropanoic acid.

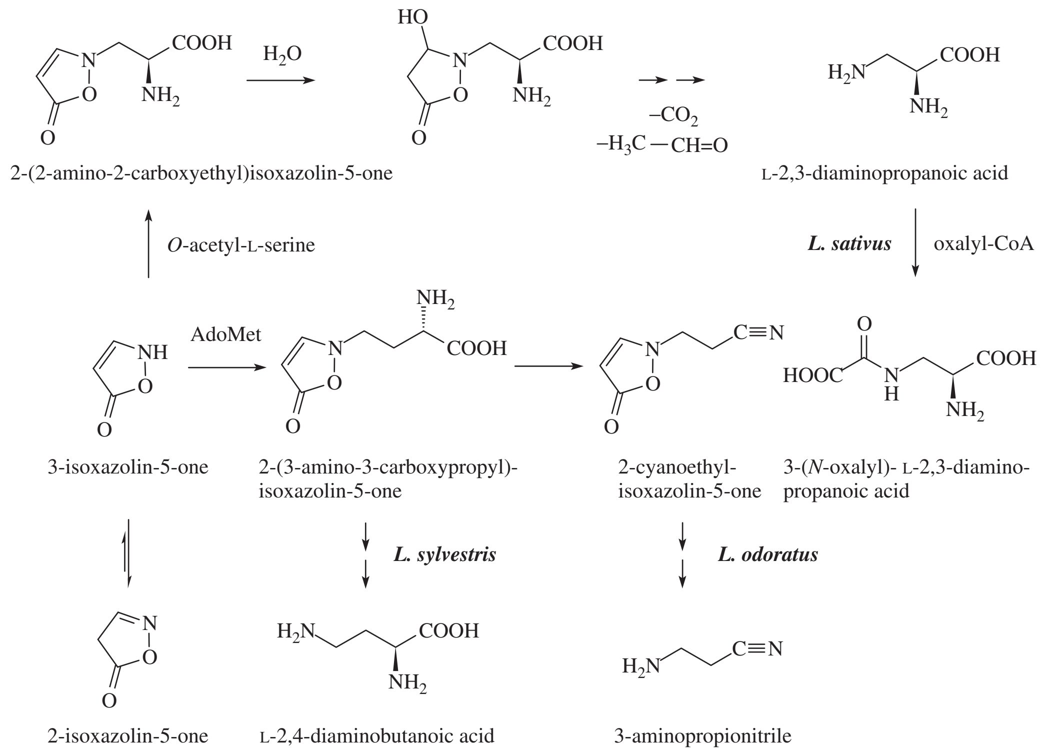


Figure 10.15 Biosynthesis of lathyrogens in vetchings.

Arginine analogues

A number of legume plants, especially seeds of tropical legumes, but also seeds of common vetch species, contain structural analogues of arginine, which may interfere with its metabolism (metabolism of ornithine) in the urea cycle. An example of these amino acids is a unique amino acid L-canavanine (see **2-44**), which was first isolated from Jack beans (*Canavalia ensiformis*), probably native to South America, which is cultivated for its edible pods and seeds, or as a reclamation plant and for green manure. The concentration in jack bean seeds is around 50 g/kg of dry matter. Later, this amino acid was identified in a number of other legumes.

An unusual amino acid is L-indospicine (see **2-45**) occurring in seeds of indigo shrubs (*Indigofera hendecaphylla*, syn. *I. spicata*) of the Fabaceae family. Its amount in these seeds is about 20 g/kg (dry weight). Indospicine is accompanied by canavanine (9 g/kg), which is also toxic. Another analogue of arginine is homoarginine, which occurs in the seeds of vetchlings and vetches together with L-4-hydroxyarginine and homocitrulline (see **2-42**). Seeds of red pea contain homoarginine at a level of about 12 g/kg of dry matter.

Sulfur and selenium amino acid analogues

Some sulfur amino acids or their selenium analogues may also exhibit toxic effects in livestock. An example of a sulfur-containing amino acid that is toxic to cattle, sheep, goats and other ruminants is a derivative of L-cysteine, *S*-methyl-L-cysteine sulfoxide, which is known as methiin (**2-23**). Methiin commonly occurs in forage brassicas, such as forage rape (*Brassica napus*), leaf turnips or forage brassica hybrids (*B. campestris*), kale (*B. oleracea*), turnips (*B. rapa*) and swedes (*B. napobrassica*) (see Section 2.2.1.2.2). The content of methiin in forage brassicas varies over a wide range of about 3–14 g/kg of dry weight, depending on the variety, the time of harvest and other factors. For example, its content in forage rape is commonly around 0.1 g/kg of dry weight, but may also reach 5–7 g/kg. Methiin also occurs in cabbage and broccoli, where its content is about 0.2 g/kg, in Brussels sprouts it is 0.7 g/kg, and is also found in onion and garlic, which contain methiin at concentrations of about 1.6 and 3 g/kg of fresh weight, respectively.

An analogue of L-cystine is 3,3′-(methylendithio)di-L-alanine, known as L,L-djenkolic acid (see **2-32**), which may cause poisoning if consumed by humans or livestock. It occurs, for example, in mildly toxic jengkol (djenkol) beans (*Archidendron pauciflorum*, syn. *Pithecellobium lobatum*, Fabaceae). Despite its strong smell, the beans are a popular food in Southeast Asia. Djenkolic acid also occurs in *Leucaena* forage legumes, such as *L. esculenta* (Mimosaceae) at about 2 g/kg and in seeds of trees and shrubs of the genus *Abarema* (syn. *Pithecolobium*), such as *A. undulatum* (Fabaceae) at a concentration of 20 g/kg dry weight.

A cause of livestock poisoning can also be selenium analogues of sulfur amino acids present in greater amounts in plants called Se-accumulators that grow on seleniferous soils (see Section 2.2.1.2.2).

Other toxic amino acid

Toxic effects, reflected particularly in farm animals, are exhibited by many other amino acids present in higher plants. Less frequently,

these amino acids may occur as toxic substances in human nutrition. Toxic amino acids also contain higher fungi (see Section 10.3.3.9).

Also toxic is the common amino acid L-3,4-dihydroxyfenylalanine (dopa), which occurs in relatively large quantities (about 50-60 g/kg fresh weight) in the immature seeds of faba beans (*Vicia faba*, Fabaceae), which are also involved in the formation of favism (see Section 10.3.1.3). The mature seeds contain about ten times lower levels of this amino acid. Similar concentrations of dopa also occur in the sub-tropical and tropical legume known as velvet bean (*Mucuna pruriens*). Toxic for ruminants in higher amounts are the proteinogenous amino acid L-tryptophan and its degradation product 3-methylindole (skatole, see **2-112**), respectively. Skatole is produced from tryptophan in the rumen as a product of decomposition by bacteria of the genus *Lactobacillus*.

An important amino acid toxic to livestock is L-mimosine, β-(3-hydroxy-4-pyridone-1-yl)-L-alanine (see **2-62**). It occurs in plants of genera *Leucaena* and *Mimosa* of the family Mimosaceae. Its quantity in the fodder plant *L. leucocephala* can reach up to 30–50 g/kg of dry matter.

An unusual toxic amino acid containing a cyclopropane ring in the molecule is hypoglycin A, L-3-(methylencyclopropyl)alanine. Hypoglycin occurs as a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-diastereoisomers in immature fruits (pulp and seeds) of ackee (akee) fruit (*Blighia sapida*) from the family Sapindaceae, originating from the island of Jamaica. It has hypoglycaemic effects but also exhibits other toxic effects. In immature seeds, the content of hypoglycin A is 1000–1110 mg/kg, but at maturation decreases to less than 100 mg/kg. Hypoglycin B is a less toxic γ-glutamyl derivative of hypoglycin A, which is only present in the seeds of the fruit. For example, the US Food and Drug Administration and Health Canada have set the maximum permissible level for hypoglycin A to 100 mg/kg.

10.3.3.9.2 Reactions and changes

Toxic amino acids undergo similar reactions and changes as the other amino acids. Compounds with a lathyrogenic effect also occur in protein isolates obtained from seeds of vetchlings and vetches, but their content is reduced by 50–85% during protein isolation. Reduction of lathyrogens can also be achieved by various culinary practices. The amount of β-*N*-oxalylaminoalanine may be reduced by up to about 50% by cooking, and by up to 95% by seed soaking, cooking under pressure and subsequent fermentation. The detoxification partly consists in the conversion of the toxic β-form to the non-toxic α-form (Figure 10.14).

Some toxic amino acids can be partially removed from food or feed by leaching. For example, the content of canavanine in Jack bean seeds can be reduced from 50 to 8 g/kg by soaking, depending on the ratio of water to extracted material and temperature. The procedure for the preparation of immature seeds of ackee fruit is based on the removal of the pericarp and cooking, which reduces the hypoglycin concentration to an acceptable level of about 1 g/kg.

10.3.3.9.3 Biological effects

Lathyrisms, and correspondingly neurolathyrism, occurs in the poorer sections of the population, (particularly in certain parts of India, Bangladesh, Ethiopia and Nepal), after approximately 3–6 months' consumption of food that contains more than two thirds of the components originating from seeds of vetchlings. Cases of neurolathyrism have also been reported in other countries, including some in Europe. Symptoms of this disease are a neurodegenerative muscular rigidity and weakness of the lower limbs, which may extend to their paralysis. In extreme cases, the disease ends in death. This disease affects more young men than women.

The lower homologue of lysine and ornithine, 2,4-diaminobutanoic acid, interferes (like the arginine analogue) with reactions of the ornithine cycle, thereby increasing the ammonia content in the blood and brain. 2,3-Diaminopropanoic acid acts as an analogue of glutamate in the nervous system.

Canavanine and other arginine analogues (indospicin and homoarginin) are not very important in human nutrition, but the crops in which they are present are important in animal nutrition. Poisoning is primarily a manifestation of growth retardation. Most sensitive to this are poultry (and other birds), which do not have the ornithine cycle. Canavanine blocks the binding of arginine (as an agonist) at a receptor molecule. By acting as an antagonist of arginine, canavanine inhibits the growth and development of other organisms, and thus has an allelochemical effect. Toxicity is based upon its degradation to canaline (by arginase) and reaction of canaline with aldehydes, for instance with pyridoxal 5′-phosphate in molecules of decarboxylases and aminotransferases, which yields stable oximes. Canavanine in mammals is partially degraded. Indospicin additionally has hepatotoxic and teratogenic effects.

Kale poisoning, or a severe haemolytic anaemia in ruminants, was discovered in cattle in Europe in the 1930s, but its link to the degradation product of methiin – dimethylsulfide – was only discovered about 35 years later. The toxic agent is dimethyldisulfide, arising as a product of methiin degradation by intestinal microflora. Dimethyldisulfide oxidises glutathione in red blood cells, the function of which is to protect haem from oxidation to haematin. In humans and other monogastric animals, such symptoms were not demonstrated.

Djencolic acid affects kidney function due to precipitation of amino acids in body fluids. Symptoms of djenkol bean poisoning (djenkolism) are nausea, vomiting, bilateral loin pain, urinary obstructions, such as haematuria (presence of blood in the urine) and oliguria (low urinary out-put, less than 500 ml in every 24 hours).

The side effects of higher amounts of dopa in humans have been associated with dizziness, staggering, increased heart rate, vomiting and psychiatric disturbances, which are consistent with its role of a precursor of the neurotransmitter dopamine. In pigs, dopa in feeds results in reduced intake and depressed weight gains. In ruminants, however, the seeds of *Vicia* and *Mucuna* have been used without apparent ill effects.

Higher amounts of tryptophan (its degradation product skatole) in feeds of ruminants may cause acute pulmonary oedema (a build-up of fluid in the spaces outside the blood vessels of the lungs) and emphysema (a chronic respiratory disease causing a decrease in lung function and often breathlessness).

Feeding crops with a higher content of mimosine, which is a thyreotoxic amino acid, is manifested by reduction of weight gain, the animals lose hair (mimosine acts as an epilator) and ophthalmic

catarrh and goitre appear. The strumigene is 3,4-dihydroxypyridine formed from mimosine degradation in the rumen. Mimosine poisoning can lead to the death of affected animals. Mimosine also has teratogenic effects. It is excreted from the body in the urine, either freely or after decarboxylation as mimosinamine.

Hypoglycin causes hypoglycaemia (low blood sugar level that is below a level necessary to properly support the body's need for energy and stability throughout its cells) and also has other toxic effects known as Jamaican vomiting sickness. Acute toxic effects are manifested mainly in malnourished subjects and by strong vomiting in children. The toxic substances are hypoglycin metabolites, which are (3*R*)- and (3*S*)-2-methylenecyclopropylacetic acids (see Section 2.2.1.2.1). Symptoms of poisoning, known as toxic hypoglycaemic syndrome, occur 6–48 hours after ingestion of seminal immature follicles. The poisoning is manifested by vomiting, drowsiness, fatigue and hypoglycaemia. The toxin (the active metabolite is methylencyclopropylacetyl-CoA) interferes with the metabolism of branched-chain amino acids, irreversibly binds to FAD and inhibits acyldehydrogenases acting in β-oxidation of fatty acids.

10.3.3.10 Biogenic amines and polyamines

Biogenic amines are a group of aliphatic, aromatic or heterocyclic bases derived from amino acids, which exhibit a variety of biological effects, as they perform different functions in animal and plant tissues. Some biogenic amines are building materials for the biosynthesis of phytohormones of the auxin group, plant protoalkaloids (such as hordenine and gramine), true alkaloids and other secondary plant metabolites. In animal tissues they have the function of tissue hormones (e.g. histamine) and are precursors of adrenal hormones (catecholamines).

Biogenic amines are formed from amino acids by the action of carboxy-lyases (decarboxylases containing as a cofactor pyridoxal 5′-phosphate), or arise from amino acids and carbonyl compounds by the action of transaminases (see Section 8.2.10.1.2). The so-called endogenous biogenic amines are the products of metabolism and at low concentrations are natural components of almost all foods. Exogenous biogenic amines are formed in foods as a result of microbial contamination and fermentation processes.

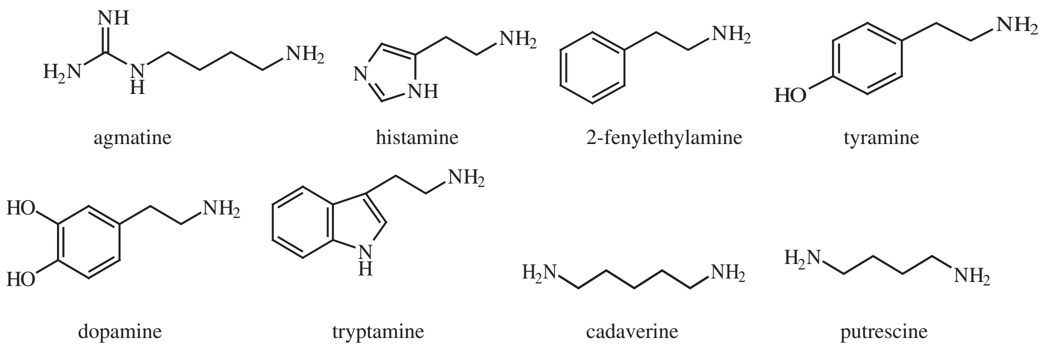
10.3.3.10.1 Structure, nomenclature and occurrence

Decarboxylation of arginine (by arginine decarboxylase) yields (4--aminobutyl)guanidine known as agmatine, histidine gives rise to 2-(1*H*-imidazol-4-yl)ethanamine known as histamine (histidine decarboxylase), phenylalanine gives phenylethan-2-amine also known as 2-phenylethylamine or phenethylamine (phenylalanine decarboxylase), tyrosine gives 4-(2-aminoethyl)phenol known as tyramine (tyrosine decarboxylase) and from 3,4-dihydroxyphenylalanine (dopa) and tryptophan (aromatic amino acid decarboxylase, also known as dopa decarboxylase or tryptophan decarboxylase) are formed 4-(2-aminoethyl)benzene-1,2-diol (dopamine) and 2-(1*H*-indol-3-yl)ethanamine (tryptamine), respectively. Decarboxylation of lysine (lysine decarboxylase) provides 1,5-diaminopentane (cadaverine), and ornithine (formed from arginine by the action of arginase) produces 1,4-diaminobutane (putrescine) by ornithine decarboxylase (**10-193**).

Putrescine, which is produced by decarboxylation of ornithine, and also arises from agmatine with catalysis by agmatinase, becomes the starting compound for the biosynthesis of spermidine and spermine. These reactions are catalysed by spermidine synthase and spermine synthase, respectively, involving *S*-adenosyl-L-methionine (for short AdoMet or SAM), and take place from bacteria to mammals (Figure 10.16). *S*-Adenosyl-L-methionine amide (dSAM) formed by decarboxylation of SAM provides trimethylene amine residue for this biosynthesis, which yields *S*-methyl-5′-thioadenosine (MTA).

Biologically active polyamines putrescine, spermidine and spermine, differ from the traditional group of biogenic amines. Spermidine and spermine arise under specific conditions and they also have different biological effects in comparison with the 'classical' biogenic amines, which are mainly histamine, tyramine, 2-phenylethylamine, putrescine, cadaverine, tryptamine and agmatine.

In the transformation of biogenic amines to other biologically active products, a number of different oxygenases, methyltransferases and other enzymes take part (Figure 10.17). Oxidation of tyramine yields 4-(2-amino-1-hydroxyethyl)phenol, known as octopamine, first demonstrated in *Octopus* species, which acts as a neurohormone and neuromodulator in invertebrates. Methylation



10-193, structures of biogenic amines

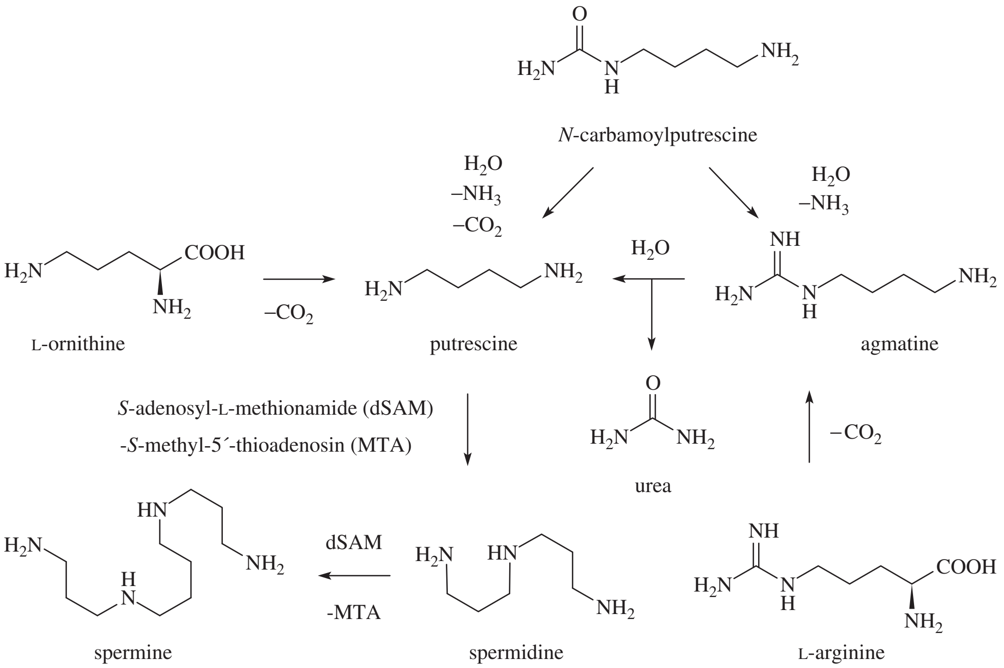
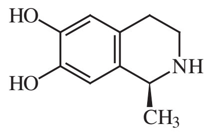


Figure 10.16 Biosynthesis of spermidine and spermine.

of tyramine gives rise to *N*-methyltyramine, which is a precursor of predominant bitter orange (*Citrus aurantium*) protoalkaloids (tyramine, *N*-methyltyramine, octopamine, hordenine and synephrine). The total amount of bitter orange biogenic amines ranges from about 10 to 80 g/kg. The main component is synephrine (8.8–71.5 g/kg). Synephrine also occurs in sweet oranges at low concentrations (about 13–35 mg/l in juice), which are, however, one of the main synephrine sources in a normal human diet. Hordenine occurs naturally in a variety of plants, taking its name from barley (*Hordeum*). In barley, hordenine levels reach a maximum within 5–11 days of germination, being about 20–30 mg/kg in malt and 12–24 mg/l in beer.

Oxidation of dopamine provides catecholamines of animals, such as adrenal hormone (*R*)-norepinephrine (also known as L-norepinephrine or noradrenaline), methylation of which yields another adrenal hormone (*R*)-epinephrine (L-epinephrine or adrenaline). Oxidation of dopamine via dopachrom leads to melanine pigments (see Section 9.3.1.1), and reaction with acetaldehyde yields salsolinol (6,7-dihydroxy-1,2,3,4-isoquinoline), which is, together with dopamine and epinephrine, the major metabolite in bananas. It is biosynthesised by non-enzymatic Pictet–Spengler condensation from dopamine and acetaldehyde, which is enzymatically generated from ethanol during the post-climacteric phase in banana (Figure 10.17). In dried banana chips, both (+)-(*R*)-salsolinol (**10-194**) and (–)-(*S*)-salsolinol occur at the 40 mg/kg level as a racemic mixture. In plants, catecholamines have a role in protection against predators and act in plant growth regulation. For example, they inhibit oxidation of phytohormone 3-indolylacetic acid called auxin (**10-85**), affect the activity of phytohormones known as gibberellins and interfere with ethylene biosynthesis (Figure 2.1).



10-194, (*R*)-salsolinol

The oxidation of tryptophan yields serotine, decarboxylation gives tryptamine, the oxidation of which leads to the hormone serotonine, from serotonine arises *N*-acetylserotonine (by the action of *N*-acetyltransferase), which is the precursor of hormone melatonin, which arises by the action of hydroxyindol-*O*-methyltransferase (Figure 10.18). An overview of biogenic amines occurring in foods, their precursors, biological activities and the main products of their transformation is shown in Table 10.22.

Biogenic amines are present in almost all foods as normal products of metabolism. At higher levels they are found in fermented products (such as cheeses, durable sausages, beer, wine and sauerkraut), where they are formed by microbial activities. Contaminating microflora primarily causes their presence in fish and meat during storage. High concentrations of biogenic amines occur in foods, such as vegetables, fruits and mushrooms, in advanced stages of spoilage under improper storage conditions.

Biogenic amines with more significant adverse effects on human health are histamine, tyramine and 2-phenylethylamine. The increased content of diamines putrescine and cadaverine serves primarily as an indicator of deficiencies in processing technology

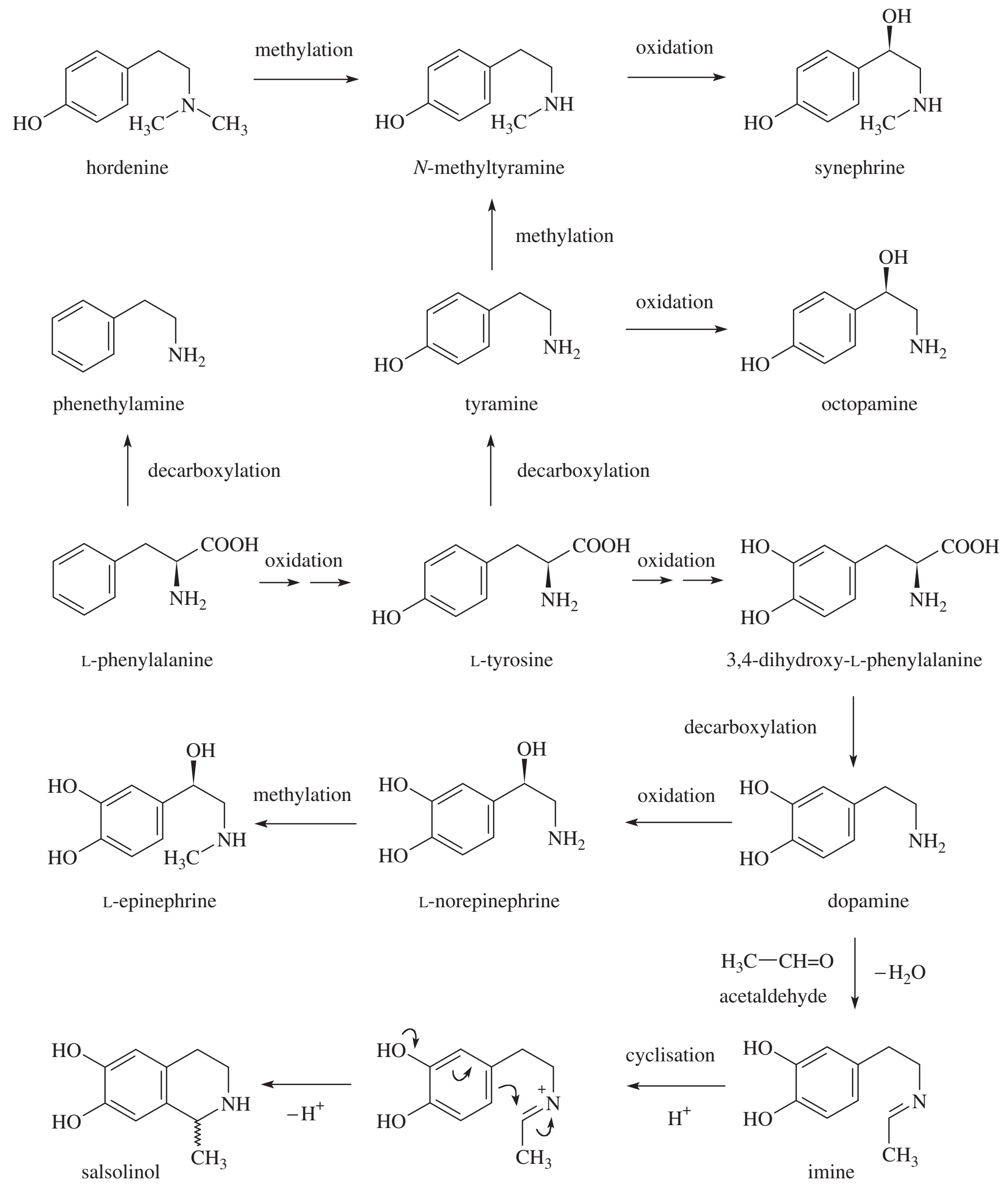


Figure 10.17 Formation of catecholamines and other metabolites of biogenic amines.

and storage of foods and food raw materials. The occurrence of tryptamine and agmatine is generally low and is not associated with adverse effects on human health. An overview of microorganisms involved in the production of biogenic amines in various foods is shown in Table 10.23. The contents of the main biogenic amines in some common foods are given in Table 10.24 (considerable variation in their contents is characteristic, as they depend on a number of factors).

Foods of animal origin

The main biogenic amines of meat, fish and cheese are histamine, cadaverine, putrescine and tyramine. During meat storage, the content of biogenic amines increases due to enzyme activity of microflora, therefore the content of some biogenic amines can be used as an indicator of meat freshness. Fresh pork meat contains, for example, up to 7 mg/kg of cadaverine and putrescine, while the rotten meat content of these two biogenic amines is 60 mg/kg and more. Cooking has relatively little influence on the content of biogenic amines, and their content only decreases by partial degradation and leaching, which is higher in pork meat. The level of the polyamines spermidine and spermine during thermal processing of meat and offal decreases by several tens of per cent, while higher decreases occur during baking and frying than during cooking or stewing.

The content of biogenic amines increases during the production of fermented sausages and ripening of cheeses. This increase is especially noticeable in the early stages of fermentation and is dependent

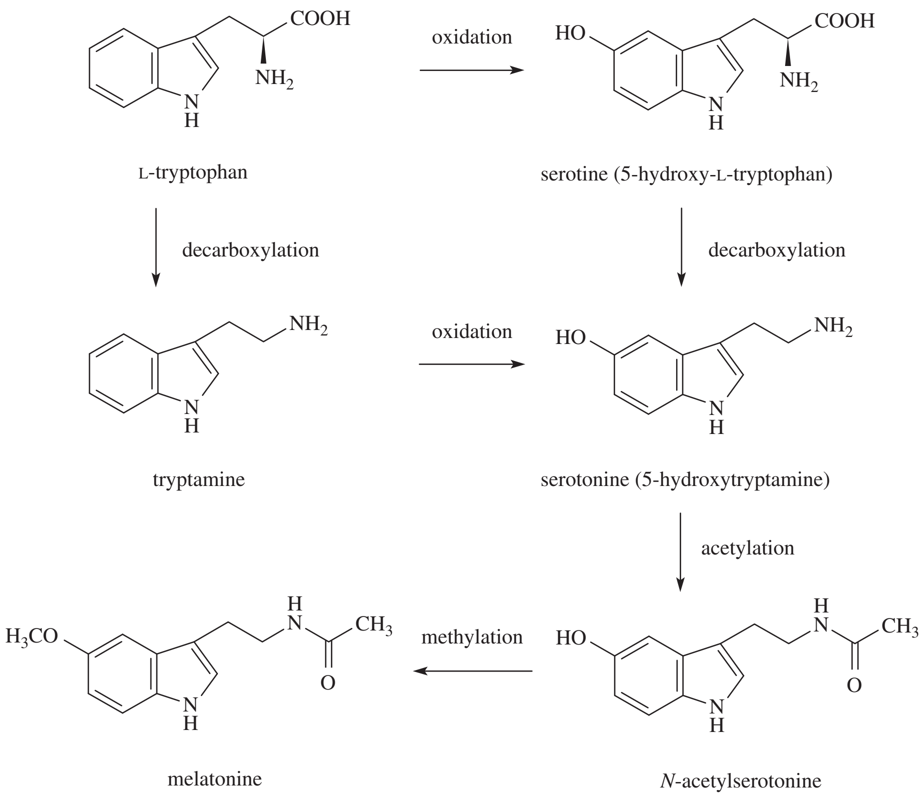


Figure 10.18 Decarboxylation and other reactions of tryptophan.

Table 10.22 Biogenic amines, their precursors, transformation products and biological activities.

Biogenic amine	Original amino acid	Other products	Biological ativity
Histamine	Histidine		Local tissue hormone, reduces blood pressure, effect on gastric juice secretion, participation in anaphylactic shock and allergic reactions
Cadaverine	Lysine		Stabilisation of macromolecules (nucleic acids), subcellular structures (ribosomes), stimulation of cell differentiation, vegetable hormone
Putrescine	Arginine *via* ornithine or citrulline	*N*-Methylputrescine, spermidine, spermine	Stabilisation of macromolecules (nucleic acids), subcellular structures (ribosomes), stimulation of cell differentiation, vegetable hormone
Agmatine	Arginine	Putrescine, *N*-methylputrescine, spermidine, spermine	Stabilisation of macromolecules (nucleic acids), subcellular structures (ribosomes), stimulation of cell differentiation, vegetable hormone
Phenethylamine	Phenylalanine	Tyramine, dopamine, epinephrine, norepinephrine	Neuromodulator, neurotransmitter in mammalian central nervous system
Tyramine	Tyrosine	Dopamine, epinephrine, norepinephrine, synephrine, hordenine	Precursor of dopamine, local tissue hormone, increases blood pressure, effect on smooth muscle contraction
Dopamine	Dopa	Norepinefrine, epinefrine	Mediators of sympathetic nerves
Tryptamine	Tryptophan	Serotonine, melatonine	Locan tissue animal and plant hormones (catecholamines), effect on blood pressure, intestinal peristalsis, mental functions

Table 10.23 Important microorganism producing biogenic amines.

Food	Microorganismd	Amines produced
Fish	*Morganella morganii*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Proteus mirabilis*, *Proteus vulgaris*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Bacillus* spp., *Staphylococcus xylosus*	Histamine, tyramine, cadaverine, putrescine, agmatine
Cheeses	*Lactobacillus buchneri*, *L. bulgaricus*, *L. plantarum*, *L. casei*, *L. acidophilus*, *L. arabinosae*, *Streptococcus faecium*, *S. mitis*, *Bacillus macerans*, *Propionibacterium* spp.	Histamine, cadaverine, putrescine, tyramine, tryptamine
Meat and meat products	*Pediococcus* spp., *Lactobacillus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Micrococcus* spp., *Enterobacteriaceae*	Histamine, cadaverine, putrescine, tyramine, phenethylamine, tryptamine
Fermented vegetables	*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Pediococcus* spp.	Histamine, cadaverine, putrescine, tyramine, phenethylamine, tryptamine
Fermented soya products	*Rhizopus oligosporus*, *Trichosporon beigllii*, *Lactobacillus plantarum*	Histamine, cadaverine, putrescine, tyramine, tryptamine

on the type of microorganisms. The formation of biogenic amines in durable salami and cheeses is caused by microorganisms of the processed raw material (Table 10.25). In rare cases, products may contain 100–1000 mg/kg of histamine, up to 580 mg/kg of putrescine, up to 90 mg/kg spermidine and up to 100 mg/kg spermine. The currently used starter cultures should have very low or negligible activity of amino acid decarboxylases.

Biogenic amines occur in fresh fish meat in small amounts. For example, tuna meat contains 0–10 mg/kg of histamine and 0–2 mg/kg of tyramine, but the content of biogenic amines increases with improper storage. During storage of fish at temperatures around 0 °C and lower, biogenic amines are formed in almost negligible quantities. At higher temperatures, the microflora in fish produces mainly histamine and tissues of Scrombroidae fish, which include tuna and mackerel, can contain up to 3000 mg/kg (mackerel) or even 8000 mg/kg (tuna) of histamine. The optimum temperature for histamine formation is 5–38 °C, but it depends mainly on the type of contaminating microflora. This is due to high levels of free, easily accessible histidine in the muscles of these fish species. In relatively high amounts other biogenic amines also arise, such as tyramine, cadaverine and putrescine. The minor biogenic amine agmatine is usually found in meat and fish in amounts of 1–3 mg/kg. High concentrations of agmatine, however, are found in some species of shellfish and dried fish. For example, the fresh Japanese abalones (*Haliotis sieboldii*) contain 40–200 mg/kg of agmatine. Its level in dried fish is up to 650 mg/kg.

Ripening of cheeses leads to a significant formation of biogenic amines only in plants with poor hygiene levels, where it is caused by contaminating microflora. With good technology and following good hygienic principles, even long-term ripened cheeses contain only small amounts of biogenic amines and polyamines, of which spermidine is present in the highest amount. The situation is different in cheeses made from unpasteurised milk (mainly milk of sheep and goats) that are often produced in small factories with hygiene deficiencies, even in developed countries.

The removal of already formed biogenic amines in foods is very difficult. A decrease in their concentration can be achieved by using diaminooxidase, but in practice it is impossible to use this enzymatic method of decontamination. Partial reduction of amines also occurs in heat-treated products, where biogenic amines can react with reducing sugars and their decomposition products in the Maillard reaction. The best way of producing foods containing small amounts of biogenic amines is the observance of such processes and hygienic conditions, which prevent their formation.

Foods of plant origin

Biogenic amines likewise occur as natural constituents in foods of plant origin. In some plants different derivatives of biogenic amines, which are commonly classified as protoalkaloids, are found in significant amounts. The main biogenic amine in fruits and vegetables is tyramine; many other biogenic amines are present in smaller quantities, often as conjugates with cinnamic or fatty acids. The main biogenic amine of bananas is tyramine, followed by phenylethylamine, histamine, dopamine, serotonine and norepinephrine. Higher amounts of putrescine occur in citrus fruits. In the United States, for example, oranges and grapefruits (including juices) represent the largest diet item in the intake of polyamines, which is due to the presence of putrescine. A less common biogenic amine is synephrine, which is derived from tyramine (Figure 10.17). It occurs almost exclusively in bitter oranges (*Citrus aurantium*, Rutaceae), and is also used in slimming diets. In spinach leaves, for example, free histamine occurs at a concentration of approximately 60 mg/kg, and also *N*-methylhistamine, *N*-acetylhistamine and histamine amides with various carboxylic acids.

Examples of conjugates of biogenic amines (**10-193**) with phenolic acids, which exhibit fungicidal activity, are hordatines (**10-195**), dimers of *p*-coumaroylagmatine, occurring in germinating barley. Wheat, for example, contains amides of 2-hydroxyputrescine along with ferulic and *p*-coumaric acids, which arise as phytoalexins.

Table 10.24 Main biogenic amines and polyamines contents in foods.

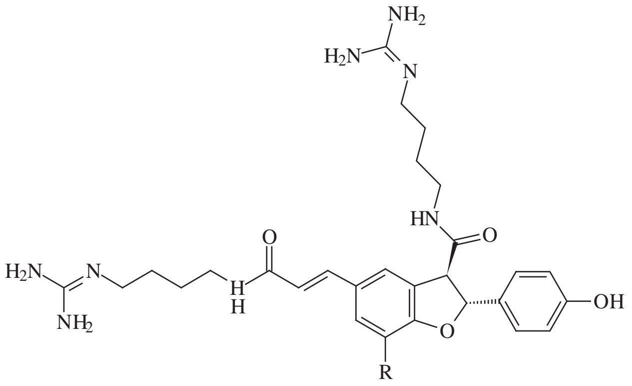
	Content (mg/kg) (or mg/l)[a]									
Foods	**Histamine**	**Cadaverine**	**Putrescine**	**Spermidine**	**Spermine**	**Agmatine**	**Phenethylamine**	**Tyramine**	**Tryptamine**	**Serotonine**
Meat										
Pork	0–45	0–171	Tr–702	Tr–5	5–40	–	–	1–35	1–48	–
Beef	0–217	0–27	Tr–26	Tr–5	5–40	2–112	–	Tr–61	–	–
Chicken	1	9	Tr–10	5–10	20–60	–	Tr	23	–	–
Meat products										
Ham	1–271	Tr–97	Tr–20	Tr–8	20–60	–	Tr–215	Tr–618	8–67	–
Bacon	15	Tr–1	Tr–8	2–42	1–212	–	–	1–3	4	–
Sausages	Tr–550	Tr–787	1–396	Tr–10	10–60	–	0–696	0–1 240	0–29	
Fish										
Tuna	Tr–8 000	Tr–447	Tr–200	1–10	2–35	–	Tr–45	Tr–1 060	–	–
Mackerel	Tr–3 000	Tr–226	Tr–40	2–4	Tr–8	–	Tr–126	Tr–75	–	–
Cheeses										
Soft cheese	0	0–1.5	0–3.1	0–0.8	0–1.1	0	0	0–0.6	0	–
Hard cheese[b]	0–301	0–710	0–612	0–43	0–19	0–22	0–32	0–301	0–45	–
Hard cheese[c]	0–609	0–389	0–670	0–40	0–22	0–27	0–30	0–609	0–34	–
Cheddar	0–1 300	0	1–996	–	–	–	0–303	0–1500	0–300	–
Emmental	Tr–2000	0–460	1–130	–	–	–	0–490	1–1000	0–210	–
Gouda	0–850	1–140	1–200	–	–	–	0–46	0–670	10–200	–
Edam	0–88	Tr	Tr	–	–	–		Tr–320		–
Roquefort	0–4100	42–905	44–830	–	–	–	10–25	Tr–1350	10–1100	–
Fruits										
Bananas			5	10	Tr	–	–	7–95	–	12–78
Pineapples	2–65	–	–	–	–	–	–	0–4	–	–
Oranges	–	–	95–150	Tr–10	Tr	–	–	1–10	Tr	–
Grapefruits	–	–	20–90	2–15	Tr	–	–	0–1400	–	–
Vegetables										
Spinach	60	–	Tr–120	1–15	Tr–4	–	–	0–680	–	–
Tomatoes	Tr–1	–	10	Tr	Tr	–	–	0–1200	4	12
Other foods										
Souerkraut	1–200	1–311	6–550	Tr–45	Tr	–	0–9	2–310	–	–
Soya sauce	0–274	–	Tr–500		–	–	Tr	Tr–882	Tr–100	–
Beer	0–22	0–40	2–15	0–7	0–4	1–41	0–8	1–68	0–5	–
Malt	1–4	–	4–10	–	–	23–117	–	9–28	–	–
Wine (red)	0–30	0–47	2–20	Tr	Tr	–	Tr	0–90	–	–
Wine (white)	0–20	3–108	1–11	Tr	Tr	–	–	Tr–212	–	–
Sherry	0–31	1	3–25	–	–	–	1	1–17	–	–
Chocolate	0–10	0–8	0	1–2	Tr–11	–	0–27	0–2	Tr–1	–

[a] Tr = traces.
[b] Produced from pasteurised cows' milk.
[c] Produced from raw sheeps' and cows' milk.

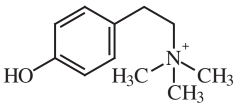
Table 10.25 Changes of biogenic amine amounts during fermentation of durable sausages.

		Content (mg/kg)				
Starting culture	**Days**	**Histamine**	**Cadaverine**	**Putrescine**	**Phenethylamine**	**Tyramine**
High activity of decarboxylases	0	3	1	1	2	2
	1	4	7	21	2	69
	2	5	35	18	9	95
	9	8	64	15	11	142
	21	6	84	13	11	120
Low activity of decarboxylases	0	3	1	1	2	2
	1	3	1	1	2	2
	2	3	1	1	2	5
	9	4	1	2	2	20
	21	3	1	3	2	21

Potent antioxidants comprise the polyamine conjugates, *N*,*N*′-dicoumaroylputrescine, *N*-*p*-coumaroyl-*N*′-feruloylputrescine and *N*,*N*′-diferuloylputrescine that were isolated from maize bran. In viral infections, tobacco produces caffeoylputrescine and other phytoalexins. In germinating barley (especially in the root) a derivative of tyramine known as hordenine (Figure 10.17) and the corresponding quaternary base candicine (**10-196**) are found.



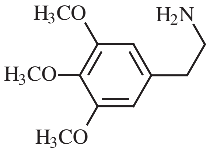
10-195, hordatine A, R = H
hordatine B, R = OCH_3



10-196, candicine

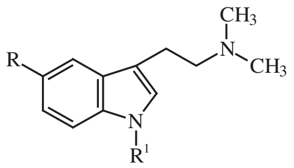
Biogenic amines, including polyamines, also occur in beer, where they are produced by contaminating lactic acid bacteria. Their concentrations are as follows: histamine <0.2–22 mg/kg, cadaverine <0.2–49 mg/kg, putrescine <0.3–31 mg/kg, spermidine <0.2–7 mg/kg, spermine <0.2–15 mg/kg, agmatine 0.5–47 mg/kg, phenethylamine <0.2–8 mg/kg, tyramine <0.3–68 mg/kg and tryptamine 0–10 mg/kg.

Several species of cacti, especially the species *Lophophora williamsii* (Cactaceae) contain protoalkaloid mescaline (**10-197**) derived from tyramine, which has hallucinogenic effects. Aztecs used these cacti in religious ceremonies.



10-197, mescaline

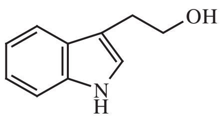
Germinating barley gives rise to protoalkaloids gramine and *N*-methylgramine (**10-198**), from tryptophan derivatives of tryptamine, which accumulate mainly in the leaves. The maximum content of gramine in leaves during the first two weeks of growth is about 900 mg/kg of fresh matter. Gramine occurs in many other monocotyledonous plants, such as grasses (Poaceae) and has the function of protection against sucking insects and plant pathogens and acts as an inhibitor of growth of certain other plants. Dimethylamine arises by partial decomposition of gramine in malt (see Section 8.2.10.1.2).



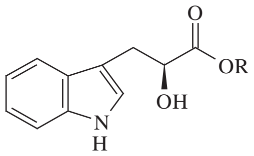
10-198, gramine, R = H, R^1 = H
N-methylgramine, R = H, R^1 = CH_3
bufotenine, R = OH, R^1 = H

Tryptamine is a precursor of a series of protoalkaloids, such as psilocin and psilocybin, which occur in hallucinogenic mushrooms (see Section 10.3.3.11.4). A protoalkaloid of certain plants,

especially mushrooms and secretions of some amphibians (frogs), is bufotenine (**10-198**), which is derived from 5-hydroxytryptamine. Tryptamine is also a precursor of plant hormones (phytohormones), a representative of which is 3-indolylacetic acid (**10-85**). A small amount of this acid is found in many foods of plant origin. In wines, for example, 3-indolylacetic acid occurs together with biosynthetic intermediates and other related compounds, such as 3-indolylethanol (**10-199**), 3-indolyl-L-lactic acid, ethyl 3-indolyl-L-lactic acid (**10-200**) and its β-D-glucoside.



10-199, 3-indolylethanol



10-200, 3-indolyl-L-lactic acid, R = H
ethyl 3-indolyl-L-lactate, R = CH_2CH_3

Phytohormones include **auxins** (such as 3-indolylacetic acid), which control cell division, germination, phototropism (the tendency of growing plant organs to move or curve under the influence of light) and other processes. The mechanism of their action is to increase the rate of RNA transcription. Another group are **gibberellins** (e.g. some terpenoids), which stimulate cell division, determine the stability of the plant shape and regulate geotropism (the downward growth of plant roots). Their mechanism of action is to stimulate the synthesis of RNA. **Cytokinins** (some isopentenyladenosines) regulate cell differentiation. Phytohormones also include **ethylene** (stimulating maturation of fruits) and **abscisic acid** (regulating aging, loss of leaves and opening of stomates).

10.3.3.10.2 Reactions and changes

Biogenic amines are reactive substances (Figure 10.19). In addition to enzymatic reactions that lead to biogenic amine derivatives and other compounds, these compounds can give rise to aldehydes by oxidative deamination. Under long-term storage or at elevated temperatures they react with triacylglycerols to form fatty acid amides. Analogously with other amino compounds, biogenic amines react with reducing sugars in the Maillard reaction yielding corresponding imines and other products. Imines also arise by oxidation of biogenic amines with hydrogen peroxide or lipid hydroperoxides. Secondary amines can react with nitrogen oxides to form carcinogenic nitrosamines. Proteins react with biogenic amines such as phenylethylamine, putrescine, histamine, tyramine and spermidine, to form β-*N*-substituted derivatives of diaminopropanoic acid. The likely mechanism of formation of these amino acid derivatives is β-elimination of cysteine residues and subsequent addition of an amine to the double bond of dehydroalanine,

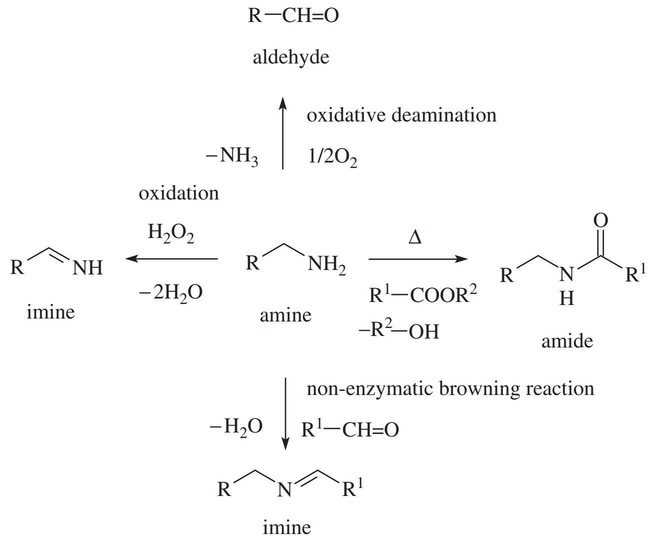
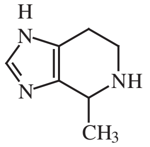


Figure 10.19 Main reactions of biogenic amines.

which arises analogously as in the case of lysinoalanine formation (see **2-121**). A Pictet–Spengler condensation reaction product of tryptamine (and tryptophan) with aldehydes in many foods yields 1,2,3,4-tetrahydro-β-carboline and β-carboline alkaloids (see Section 2.5.2.3.1). Reaction of histamine with acetaldehyde yields 4-methyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine), known as 4-methylspinaceamine, which is present in fermented foods, such as oriental sauces and some cheeses (**10-201**).



10-201, 4-methylspinaceamine

10.3.3.10.3 Biological effects

Biogenic amines (in the narrower sense, biogenic amines does not include polyamines spermidine and spermine) are indispensable for the body, but at high concentrations may act as:

- psychoactive amines
- vasoactive amines.

Psychoactive amines act as transmitters in the central nervous system, vasoactive amines act directly or indirectly on the vascular system. According to the action, vasoactive amines can be divided into:

- vasocontractibile amines (e.g. tyramine)
- vasodilating amines (such as histamine).

Symptoms of consuming high doses of biogenic amines are vomiting, difficulty breathing, sweating, palpitations, hypotension

(histamine) or hypertension (tyramine) and migraine (phenylethylamine and tyramine). Monoamine oxidase and diamine oxidase are the main enzymes that decompose biogenic amines in the gut. Spermine and spermidine are decomposed by polyamine oxidase, but polyamine oxidase-mediated catabolism of spermine and spermidine produces putrescine and toxic acrolein (Figure 10.20). The toxic effect of biogenic amines is strongly influenced by the activity of these enzymes, which may be different for different individuals and depends on many factors, such as the presence of inhibitors (certain medicines, particularly from the group of psychotropic drugs, and to a lesser extent alcohol) or potentiators. High concentrations of biogenic amines cannot be eliminated by this enzyme system. Part of its capacity is needed particularly for the detoxification of histamine and tyramine depletes putrescine and cadaverine, which do not themselves possess a health risk, but their content may be quite high in a variety of foods (Table 10.24).

When evaluating the toxic effect of biogenic amines, it is necessary to consider not only the presence of a particular amine, but also other factors, such as the amount of food consumed, the presence of other toxic substances and other issues. For this reason it is very difficult to set the levels that have adverse effects in food. No adverse health effects were observed after exposure to the following biogenic amine levels in food (per person per meal): 50 mg histamine for healthy individuals, but below detectable limits for those with histamine intolerance; 600 mg tyramine for healthy individuals not taking monoamino oxidase inhibitor (MAOI) drugs, but 50 mg for those taking third generation MAOI drugs or 6 mg for those taking classical MAOI drugs and for putrescine and cadaverine. Concentrations of histamine higher than 500-1 000 mg/kg are considered to be dangerous to humans. Increased amounts of histamine can cause anaphylactic shock (very serious allergic reaction manifested by dizziness, loss of consciousness, laboured breathing, swelling of the tongue and breathing tubes, blueness of the skin, low blood pressure, heart failure and death). Histamine poisoning (histaminosis) from marine fish and fish products in coastal countries is the most common cause of foodborne poisonings. In many countries the maximum amount of histamine and tyramine is set, but information on the toxicity of other biogenic amines is inadequate. Commission regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs sets the limits for fishery products from fish species associated with a high amount of histidine (particularly fish species of the families Scombridae, Clupeidae, Engaulidae, Coryfenidae, Pomatomidae and Scombresosidae) to 100–200 mg/kg and to 200-400 mg/kg for fishery products, which have undergone enzyme maturation treatment in brine. For example, the Decree No. 305/2004 Coll. (in the Czech Republic) set limits for histamine to 200 mg/kg in fish and fish products and to 20 mg/l for beer and wine.

As multifunctional cations polyamines spermidine and spermine perform many important biological roles. The most important of these is the participation in protein biosynthesis and biosynthesis of nucleic acids. The biological processes in animals involve polyamines from three sources: endogenous produced in cells, produced by the gastrointestinal tract microflora and foodborne polyamines. Increased intake of dietary polyamines is desirable for accelerated healing of wounds, burns, development and recovery of the intestinal mucosa and for other processes, but the dietary intake should be low in people with cancer. There is an increased level of polyamines in young, fast-growing and metabolically active tissues (such as the liver and kidney). Plant products contain more spermidine than spermine, while animal products contain more spermine than spermidine. The physiological need for polyamines is not known.

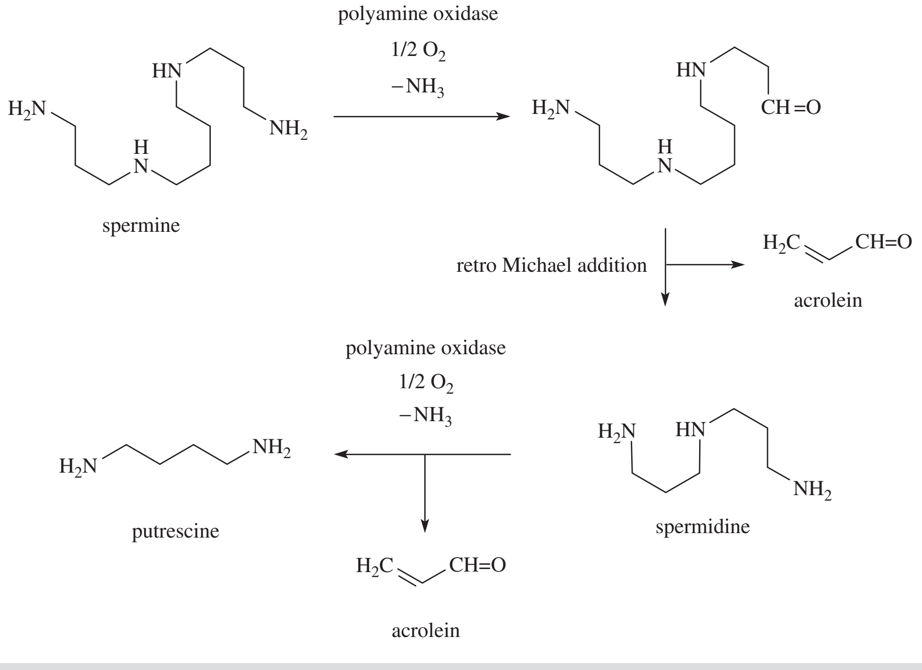


Figure 10.20 Catabolism of spermine and spermidine.

The protoalkaloid gramine, found mainly in grasses of the genus *Phalaris* (Poaceae), may cause sudden collapse and death of the grazing cattle. In sheep, the chronic poisoning is manifested by degenerative changes in the central nervous system.

10.3.3.11 Mushroom toxins

Mushrooms have inspired the cuisines of many cultures (notably Chinese, Japanese and European) for centuries and many species have been used in folk medicine for thousands of years, but approximately 70% of poisonings caused by natural substances can be attributed to mushrooms. About 5000 mushrooms species occur in nature, of which approximately 50–100 are known to be poisonous, only about ten are fatally poisonous and only 200–300 species have been clearly established to be eaten safely. However, many people are still hospitalised after eating mushrooms and several deaths are also reported annually after mushroom poisoning. Mushroom poisoning is more common in Europe than in America. The only way to be able to tell if a particular fungus is edible is to correctly identify its species.[4] There are no short cuts. Toxic substances occurring in mushrooms can be chemically divided into:

- toxic proteins
- toxic cyclopeptides
- toxic amino acid
- toxic amines, alkaloids and other nitrogenous compounds
- toxic terpenoids.

According to the severity, the most dangerous form of mushroom poisoning, which accounts for most mushroom related deaths, is that caused by cyclopeptides of toxic species of toadstools (*Amanita* spp.), called **phalloidin poisoning**, which is followed by poisoning due to cortinars (webcaps, *Cortinarius* spp.), which is called **orellanine poisoning**.

10.3.3.11.1 Proteins

Structure, nomenclature and occurrence

The toxic protein of false parasol, also known as the green-spored parasol (*Chlorophyllum molybdites*, Agaricaceae) has a relative molecular weight of 400 kDa and is composed of several subunits of molecular weights of 40–60 kDa. Bolaffinin is a toxic protein isolated from spotted bolete (*Xanthoconium affine*, syn. *Boletus affinis*, Boletaceae) containing 234 amino acid residues (molecular weight of 22 kDa) with one disulfide bridge. Bolesatin is a toxic glycoprotein with a molecular weight of 63 kDa isolated from devil's bolete (Satan's mushroom, *Boletus satanas*, Boletacae). Wood pink gill (*Entoloma rhodopolium*, syn. *Rhodophyllus rhodopolius*, Entoolomataceae) contains a toxic protein with a molecular weight of 40 kDa.

Reactions and changes

The toxic protein of false parasol is labile and is cleaved by proteolytic enzymes. Bolaffinin loses toxicity after being boiled for 15 min. Bolesatin is relatively thermostable and is resistant to hydrolysis by proteolytic enzymes.

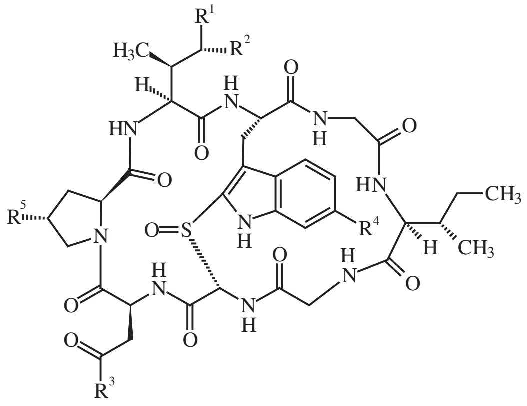
Biological effects

Toxic protein of false parasol exhibits haemolytic activity and causes poisoning, which is manifested by diarrhea and hypersensitivity to light and noise, but the poisoning results in death only in rare cases. Bolaffinin inhibits protein synthesis and damages the liver. By similar effects as lectins (cytotoxicity, inhibition of protein synthesis, see Section 10.3.3.8) manifests bolesatin.

10.3.3.11.2 Peptides

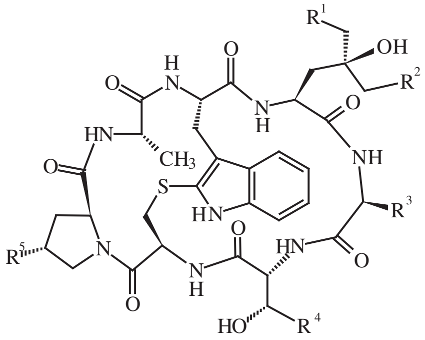
Structure, nomenclature and occurrence

The most important compounds of this group of fungi toxins are the cyclic peptides amatoxins or amanita toxins (**10-202**) and phallotoxins (**10-203**) of the death cap (*Amanita phalloides*, Amanitaceae) that cause the phalloidin poisoning. The main toxin is phalloidin



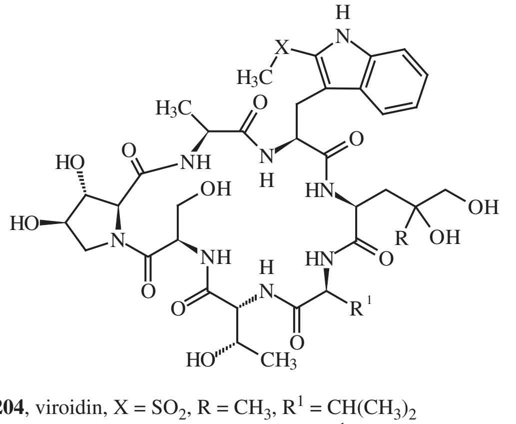
10-202, α-amanitin, $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = NH_2$, $R^4 = OH$, $R^5 = OH$
β-amanitin, $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$, $R^5 = OH$
γ-amanitin, $R^1 = CH_3$, $R^2 = OH$, $R^3 = NH_2$, $R^4 = OH$, $R^5 = OH$
ε-amanitin, $R^1 = CH_3$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$, $R^5 = OH$
amanine, $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = H$, $R^5 = OH$
amanineamide, $R^1 = CH_2OH$, $R^2 = OH$, $R^3 = NH_2$, $R^4 = H$, $R^5 = OH$
amanulline, $R^1 = CH_3$, $R^2 = H$, $R^3 = NH_2$, $R^4 = OH$, $R^5 = OH$
amanullic acid, $R^1 = CH_3$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$, $R^5 = OH$
proamanulline, $R^1 = CH_3$, $R^2 = H$, $R^3 = NH_2$, $R^4 = OH$, $R^5 = H$

[4]The taxonomy of the Fungi kingdom (the sub-kingdom Dikarya) is in a constant flux; therefore, the most recent 2007 classification adopted by a coalition of mycologists has been used. Out of seven phyla (divisions) proposed, the toxic fungi of this section belong to the phylum Basidiomycota (known as club fungi), subphylum Agaricomycotina, class Agaromycetes, subclass Agaromycetidae, which covers most of the so called higher fungi. The phylum Ascomycota (sac fungi) covers only higher fungi belonging to the subdivision Pezizomycotina, class Pezizomycetes, subclass Penzizomycodidae, order Pezizales.



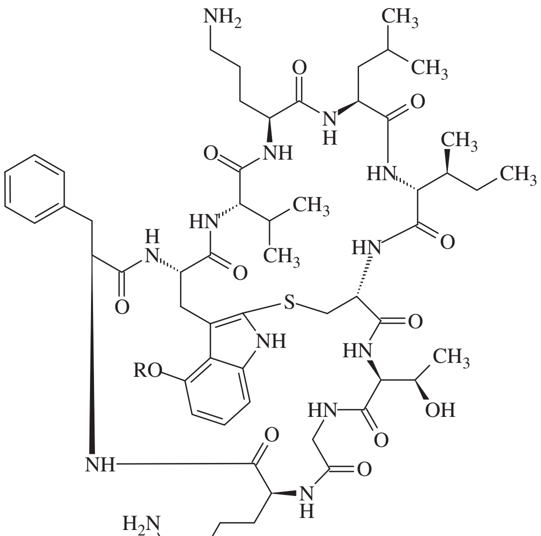
10-203, phalloidin, $R^1 = OH$, $R^2 = H$, $R^3 = CH_3$, $R^4 = CH_3$, $R^5 = OH$
phalloin, $R^1 = H$, $R^2 = H$, $R^3 = CH_3$, $R^4 = CH_3$, $R^5 = OH$
prophalloin, $R^1 = H$, $R^2 = H$, $R^3 = CH_3$, $R^4 = CH_3$, $R^5 = H$
phallisin, $R^1 = OH$, $R^2 = OH$, $R^3 = CH_3$, $R^4 = CH_3$, $R^5 = OH$
phallacin, $R^1 = H$, $R^2 = H$, $R^3 = CH(CH_3)_2$, $R^4 = COOH$, $R^5 = OH$
phallacidin, $R^1 = OH$, $R^2 = H$, $R^3 = CH(CH_3)_2$, $R^4 = COOH$, $R^5 = OH$
phallasacin, $R^1 = OH$, $R^2 = OH$, $R^3 = CH(CH_3)_2$, $R^4 = COOH$, $R^5 = OH$

(about 100 mg/kg of fresh mushroom), in comparable amounts occur α-amanitin (about 80 mg/kg) and β-amanitin (50 mg/kg), other toxins are present in smaller amounts. Amanita toxins are similarly found in several other Amanita species (*A. bisporigera*, *A. hygroscopia*, *A. ocreata*, *A. suballiacea*, *A. tenuifolia* and *A. virosa*) and some members of the genera *Galerina* (*G. autumnalis*, *G. marginata* and *G. venenata*) and *Lepiota* (*L. brunneoincarnata*, *L. chlorophyllum*, *L. helveola* and *L. josserandii*). A number of other related peptides known as cycloamanides have no biological activity. Similar structures and toxicities to those of phallotoxins are seen in virotoxins (**10-204**) present in the European destroying angel (*A. virosa*).

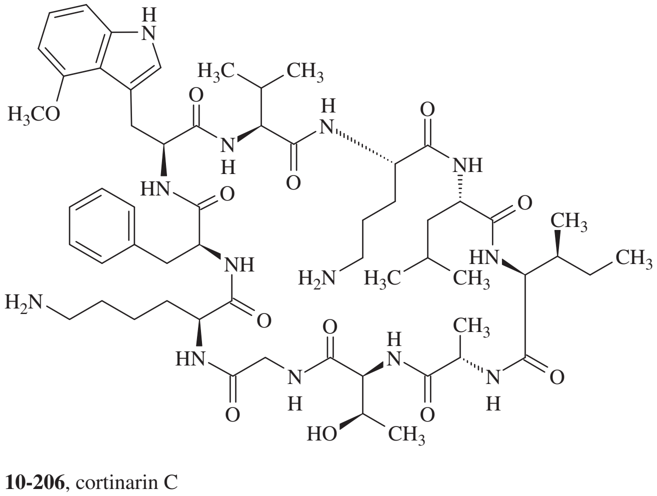


10-204, viroidin, $X = SO_2$, $R = CH_3$, $R^1 = CH(CH_3)_2$
deoxyviroidin, $X = SO$, $R = CH_3$, $R^1 = CH(CH_3)_2$
[Ala[1]]viroidin, $X = SO_2$, $R = CH_3$, $R^1 = CH_3$
[Ala[1]]deoxyviroidin, $X = SO$, $R = CH_3$, $R^1 = CH_3$
viroisin, $X = SO_2$, $R^1 = CH_2OH$, $R = CH(CH_3)_2$
deoxyviroisin, $X = SO$, $R = CH_2OH$, $R^1 = CH(CH_3)_2$

Less toxic cyclic peptides are also found in other fungi. Examples are toxins known as cortinarins (**10-205** and **10-206**) of fool's web cap (*Cortinarius orellanus*, Cortinariaceae) and other web caps that cause orellanine poisoning.



10-205, cortinarin A, $R = CH_3$
cortinarin B, $R = H$



10-206, cortinarin C

Reactions and changes

Amatoxins, phallotoxins and virotoxins are stable under all normal conditions of storage and processing, and technological processes, including heat treatment, do not reduce their high toxicity.

Biological effects

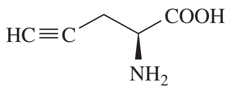
The toxicity of amatoxins is caused by the inhibition of RNA polymerase II in eukaryotic cells, toxicity of phallotoxins by selective binding of toxins to the membrane of actin (F-actin) and stabilisation of this complex. Amatoxins, of which α-amanitin is present in the highest concentration in the death cap and is responsible for its toxicity, are about 10–20 times more toxic than related phallotoxins, which exhibit toxicity only after intravenous administration. Toxicity of amatoxins and phallotoxins manifests by effects similar to cholera: gagging, vomiting and diarrhea about 20 h after eating the toadstools. These toxins are among the most toxic natural substances as they are inhibitors of important enzymes responsible for the metabolism of the major nutrients and damage the liver and kidneys. In most cases, poisoning ends with death.

Cyclic peptides cortinarins A, B and C are nephrotoxic, the poisoning manifests by acute or chronic damage to the kidneys 3–14 days after ingestion of mushrooms.

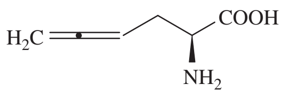
10.3.3.11.3 Amino acids

Structure, nomenclature and occurrence

As well as toxic proteins and peptides, mushrooms contain a number of free amino acids, which cause poisoning manifested by symptoms similar to those caused by the death cap toadstool. For example, (*S*)-2-amino-pent-4-ynoic acid, also known as L-2-aminopent-4-ynoic acid or L-propargylglycine (**10-207**), (*S*)-2-aminohexa-4,5-dienoic acid, also called L-2-aminohexa-4,5-dienoic acid (**10-208**), (*S*)-allylglycine or L-allylgycine (**10-209**) and related amino acids occur in some other toadstool species, such as the abrupt-bulbed lepidella (*Amanita abrupta*) and European solitary lepidella (*A. echinocephala*, syn. *A. solitaria*), where they are found together with (2*S*,4*Z*)-2-amino-5-chloro-6-hydroxyhex-4-enoic acid. In addition to 2-amino-5-chloro-6-hydroxyhex-4-enoic acid, four other chlorinated allylic amino acids have been isolated from various Amanita species. A number of other C_6 and C_7 unbranched and branched amino acids have been isolated in recent years, reflecting great variability in the metabolism of branched-chain amino acids (valine, leucine and isoleucine) in different mushroom species.



10-207, L-propargylglycine

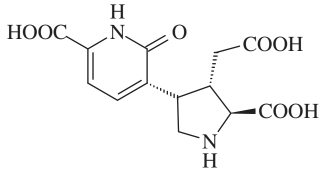


10-208, L-2-aminohexa-4,5-dienoic acid

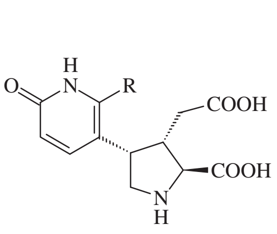
10-209, L-allylglycine

A number of other amino acids structurally related to L-glutamic acid exhibit neurotoxic (psychotropic) and other toxic effects. Glutamic acid acts as an excitatory neurotransmitter in the central nervous system of mammals. Glutamate receptors are involved in higher neural functions (such as memory and learning) and disorders (such as epilepsy). For example, the main toxic agent of the fly agaric (*A. muscaria*), which derives its name from the ability of its juice to stun and sometimes kill house flies (*Musca domestica*), and also of panther cap (*A. pantherina*) and European pine cone lepidella (*A. strobiliformis*), is (*S*)-2-amino-(3-hydroxy-5-isoxazolyl)acetic acid, known as ibotenic acid, formerly also as pantherin, agarin, α-toxin or premuscimol (**2-52**); enol and keto tautomers of ibotenic acid occur in the ratio of 96 : 4. Also present is a product of ibotenic acid decarboxylation 5-aminomethyl-3-hydroxyisoxazol, which is known as muscimol (or pyroibotenic acid or β-toxin). The ibotenic acid content in hats of toadstools is approximately 0.02%. Muscazone (2-amino-2,3-dihydro-2-oxo-5-isooxazoleacetic acid), showing antibacterial and antifungal activities, is probably formed by rearrangement of ibotenic acid. *A. strobiliformis* and Japanese mushroom *Tricholoma muscarium* (Tricholomataceae) contain another important neurotoxic amino acid (**2-53**), a dihydro analogue of ibotenic acid, 2-amino-2-(3-oxoisoxazolidin-5-yl)acetic acid, known as tricholomic acid (Figure 10.20).

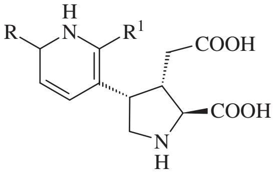
Other neurotoxins are acromelic acids (**10-210** to **10-212**). Acromelic acids A and B occur in *Clitocybe acromelalga* (Tricholomataceae) found in Japan, together with minority congeners acromelic acids C, D and E and many other related biologically active compounds.



10-210, acromelic acid A

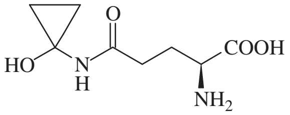


10-211, acromelic acid B, R = COOH
acromelic acid C, R = H



10-212, acromelic acid D, R= COOH, R[1] = H
acromelic acid E, R = H, R[1] = COOH

Present in the common ink cap (*Coprinopsis atramentarius*, syn. *Coprinus atramentarius*, Psathyrellaceae) and some other mushrooms, such as the fat-footed *Clitocybe* (*Clitocybe claviceps*, Tricholomataceae), sharp-scaly pholiota (*Pholiota squarrosa*, Strophariaceae) and lurid bolete (*Boletus luridus*, Boletaceae) is the toxic glutamine derivative, N^5-(1-hydroxycyclopropyl)-L-glutamine, which is known as coprine (**10-213**).



10-213, coprine

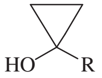
Reactions and changes

Most toxic amino acids of fungi are stable substances that do not undergo reactions leading to reduction of their toxicity. Decomposition of the original amino acid, in some cases, yields degradation products that are also toxic, such as the decarboxylation product of ibotenic acid referred to as muscimol (Figure 10.21).

Coprine is a protoxin without intrinsic toxicity, but which hydrolyses to the active toxic principle 1-aminocyclopropanol and cyclopropanone hydrate (cyclopropane-1,1-diol, **10-214**).

Biological effects

Poisoning by propargylglycine and 2-aminohexa-4,5-dienoic acid have similar symptoms as phalloidin poisoning. Although not as



10-214, 1-aminocyclopropanol, R = NH_2
cyclopropane-1,1-diol, R = OH

toxic as the death cap and the destroying angel, ingestion of the abrupt-bulbed lepidella may cause changes in liver function similar to these species. The poisoning begins with violent vomiting, diarrhea and dehydration after a delay of 10–20 h. The toxic effects of allylglycine are not yet precisely known.

Ibotenic acid is a likely carcinogen. In humans, poisoning is manifested by hallucinations. Ibotenic and tricholomic acids exhibit taste properties similar to those of monosodium glutamate (umami taste, see Section 8.3), but much more intense. Muscimol has similar effects as γ-aminobutyric acid (GABA or 4-aminobutanoic acid), which acts as an inhibitory neurotransmitter of the central nervous system.

Acromelic acids A and B also behave as neurotoxins, which act as agonists (activators) of the neurotransmitter L-glutamic acid. Their activity is manifested by special symptoms as they evoke powerful burning and reddish swellings on the legs, which last a month or longer. Deaths caused by poisoning are rare.

Many mushrooms fall into the category of those that cause only gastrointestinal-specific irritation, distress, abdominal cramping, diarrhea, nausea and vomiting. Most of these symptoms are poorly understood. To this category of mushrooms also belongs the common ink cap containing coprine, a substance that is toxic only when ingested in combination with alcohol. The degradation products of coprine, 1-aminocyclopropanol (**10-214**) and cyclopropane-1,1-diol, inhibit the enzyme aldehyde dehydrogenase, which catalyses conversion of acetaldehyde into acetic acid, which results in accumulation of acetaldehyde in the blood. Inhibition of aldehyde dehydrogenase produces a clinical syndrome similar to disulfiram (Antabuse, **10-215**) alcohol reaction, manifested by flushing,

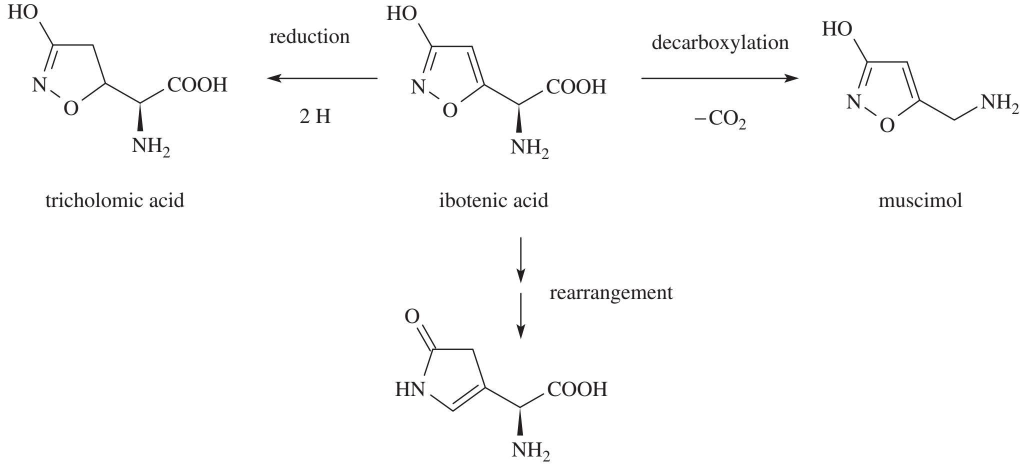
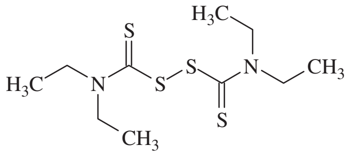


Figure 10.21 Reactions of ibotenic acid.



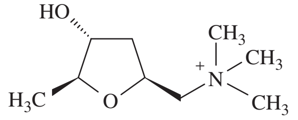
10-215, disulfiram

throbbing in the temples and usually headache starting 5–30 min after ingestion of alcohol.

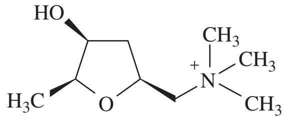
10.3.3.11.4 Amines, alkaloids and other nitrogen compounds

Structure, nomenclature and occurrence

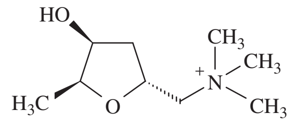
In addition to the main neurotoxin ibotenic acid, the fly agaric (*A. muscaria*) contains the toxic amine muscarine (**10-216**) in small quantities. Much higher amounts of this amine (about 500 times) occur in mycorrhizal fungi of the genus *Inocybe* that cause the **muscarinic poisoning**. The muscarine molecule has three asymmetric centres, thus can exist in eight stereoisomers, but only three muscarine isomers, epimuscarine (**10-217**), allomuscarine (**10-218**) and epiallomuscarine (**10-219**), have been found in nature.



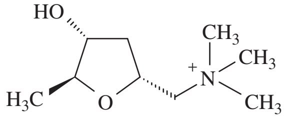
10-216, muscarine



10-217, epimuscarine

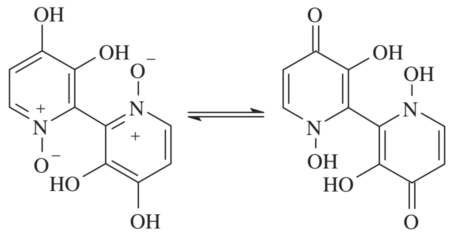


10-218, allomuscarine



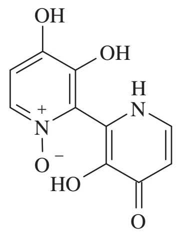
10-219, epiallomuscarine

The fungi fool's web cap (*Cortinarius orellanus*, Cortinariaceae) and deadly web cap (*C. rubellus*), and other species, that cause the so-called **orellanine poisoning**, contain a toxic pyridine alkaloid orellanine (**10-220**), its degradation products orellinine (**10-221**) and yellow orelline (**10-222**), which are produced by heating or by exposure to UV radiation. Orellanine is a highly polar compound due to tautomerism between the neutral and internal ionic form.

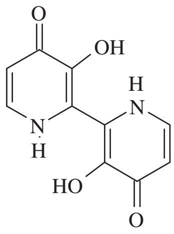


10-220, orellanine

In addition to these toxic alkaloids, these fungi contain toxic cyclic peptides cortinarins (see Section 10.3.3.11.2).

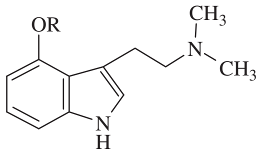


10-221, orellinine

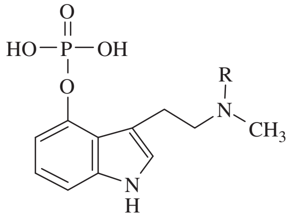


10-222, orelline

The psychedelic mushrooms of the genus *Psilocybe*, such as *P. mexicana* (Strophariaceae) and various other fungi belonging to genera *Paneolus*, *Pluteus*, *Conocybe* and some others, contain as the active component psilocin and its phosphoric acid ester psilocybin (**10-223**), which are carriers of hallucinogenic effects. Mushrooms *P. mexicana*, called *teonáncatl*, were used by Mayans for religious ceremonies over 2000 years ago. Other related hallucinogenic substances, such as baeocystin and norbaeocystin (**10-224**), occur in *P. baeocystis* of the same genus. Another hallucinogenic amine of similar structure is 3-(2-dimethylaminoethyl)-1*H*-indol-5-ol called 5-hydroxytryptamine or bufotenine (**10-198**), which is found in the false death cap, also known as citron amanita (*Amanita citrina*). The same amine also occurs in skin secretions of some toads (such as the cane toad *Bufo marinus*, Bufonidae), but also in some other animals (amphibians) and higher plants. For example, bufotenine is present at a concentration of 25 mg/l in the red latex (called *takini*) of the tree *Brosimum acutifolium* (Moraceae), which is native to the Amazon rainforest and is used in shamanic practices.



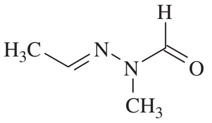
10-223, psilocin, R = H
psilocybin, R = PO_3H_2



10-224, baeocystin, R = CH_3
norbaeocystin, R = H

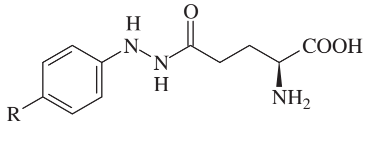
The toxic hydrazine derivative *N*-formyl-*N*-methylhydrazone of acetaldehyde, known as *N*′-ethylidene-*N*-methylformohydrazide or gyromitrin (**10-225**), occurs in the fruiting body of false

morrels (*Gyromitra esculenta*, Discinaceae) and in some other species of the phylum Ascomycota. Gyromitrin is accompanied by other *N*-formyl-*N*-methylhydrazones derived from butanal, 3-methylbutanal, pentanal, hexanal, octanal and (*E*)- and (Z)-oct-2-enal. Gyromitrin is the main toxic substance of false morels. Its concentration can generally reach up to 3600 mg/kg and the amount of gyromitrin and other hydrazones is in a ratio of about 88 : 12. The hydrazones of false morels are accompanied by *N*-formyl-*N*-methylhydrazine and *N*-methylhydrazine, the concentrations of which are 500 and 40–350 mg/kg, respectively. Parts of the gyromitrin and *N*-formyl-*N*-methylhydrazine are probably bound as glycosides linked to high molecular weight substances.



10-225, gyromitrin

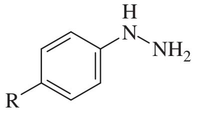
Another hydrazine of mushrooms is agaritine (**10-226**), β-*N*-[γ-L-glutamyl]-4-(hydroxymethyl)phenylhydrazine found only in fungi of the genera *Agaricus* and *Macrolepiota* (Agaricaceae), to which belong the dominant cultivated mushrooms, the common mushroom (*A. bisporus*) and the field mushroom (*A. campestris*). The best-known member of the genus *Macrolepiota* is the parasol mushroom (*M. procera*). The concentration of agaritine in fresh common mushrooms varies over a wide range (100–1700 mg/kg). For example, the average content of agaritine in the common mushroom cultivated in the Czech Republic was 272 mg/kg (165–457 mg/kg) and somewhat higher levels were found in young fruiting bodies. The agaritine content is also dependent on the growth cycle of mushrooms. Harvesting periods are repeated at 3–5 day cycles with breaks when the mushroom harvest is very small. The agaritine content is higher in fruiting bodies harvested in the latter days of the harvest cycle and lower in mushrooms grown on natural substrates than on synthetic or mixed ones. The horse mushroom (*A. arvensis*) growing in nature has a hgher agaritine content, sometimes up to 2000 mg/kg and more. Only consumption of raw mushrooms is potentially harmful, because agaritine partly decomposes under technological and culinary treatments.



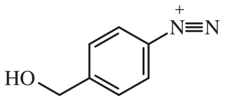
10-226, agaritine, R = CH_2OH

β-*N*-γ -L-[glutamyl]-4-(formyl)phenylhydrazine, R = CH=O
β-*N*-γ -L-[glutamyl]-4-(carboxy)phenylhydrazine, R = COOH

Agaritine may be enzymatically oxidised at the C-4 hydroxymethyl group to the corresponding formyl and carboxyl derivatives, β-*N*-[γ-L-glutamyl]-4-(formyl)phenylhydrazine and β-*N*-[γ-L-glutamyl]-4-(carboxy)phenylhydrazine (**10-226**), repectively. The presence of β-*N*-[γ-L-glutamyl]-4-(carboxy)phenylhydrazine, at a level of 40 mg/kg, has been demonstrated only in the field mushroom (*A. campestris*). Agaritine and β-*N*-[γ-L-glutamyl]-4-(carboxy)phenylhydrazine are hydrolysed to 4-(hydroxymethyl)phenylhydrazine and 4-(carboxy)phenylhydrazine (**10-227**), respectively. 4-(Carboxy)phenylhydrazine concentrations in mushrooms are about 10 mg/kg. 4-(Hydroxymethyl)phenylhydrazine is subsequently oxidised to the corresponding 4-(hydroxymethyl)benzenediazonium ion (**10-228**), the amount of which, in the basal-stalk sections of the commonly cultivated mushroom *Agaricus bisporus*, is about 1 mg/kg. In addition to these compounds, the common mushrooms contain some other precursors, metabolites and degradation products of agaritine, such as 4-carboxymethylbenzoic acid and agaritine analogues derived from 4-aminophenol, γ-glutamyl-4-hydroxybenzene, the characteristic yellow pigment agaricone (see **9-131**) of the yellow-staining mushroom *A. xanthodermus*, and other products.



10-227, 4-(hydroxymethyl)phenylhydrazine, R = CH_2OH
4-(carboxy)phenylhydrazine, R = COOH



10-228, 4-(hydroxymethyl)benzenediazonium ion

Reactions and changes

Most substances belonging to this group of toxins are stable during technological and culinary operations. Dried false morrel contains usually 1000–3000 mg/kg of gyromitrin, including its derivatives and degradation products, but drying does not have a significant influence on the gyromitrin content. For example, a prolonged drying in the air decreases the *N*-methylhydrazine content by about 30–70%. A significant decrease of hydrazine content (to 10–15% of the original value), however, occurs by leaching and volatilisation of products during cooking, and cooked mushrooms are therefore edible. The content of toxins in canned mushrooms is also much lower (6-65 mg/kg). On cooking, gyromitrin decomposes to form acetaldehyde and *N*-formyl-*N*-methylhydrazine, which is more stable than gyromitrin, but in acidic media it decomposes to *N*-methylhydrazine. Similar reactions proceed *in vivo* and 25–30% of gyromitrin may be transformed into *N*-methylhydrazine. By the action of hepatic mixed function monooxidases, however, highly toxic diazomethane and *N*-nitroso-*N*-methylformamide are produced (Figure 10.22).

The enzymatic hydrolysis of agaritine in common mushrooms by γ-glutamyl transferase gives 4-(hydroxymethyl)phenylhydrazine (**10-227**) and L-glutamic acid. The enzymatic hydrolysis occurs during storage of fresh, chilled or frozen mushrooms. During storage at temperatures of 2–12 °C, the agaritine concentration

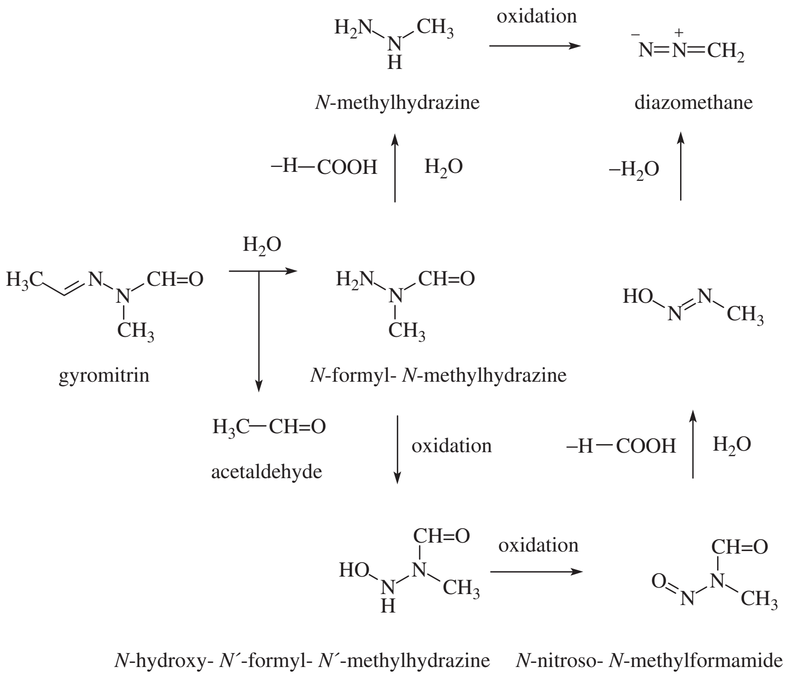


Figure 10.22 Metabolism and non-enzymatic decomposition of gyromitrin.

decreases by about 32%. Frozen mushrooms stored for 1 month and then defrosted contained about 25% of the agaritine present in fresh mushrooms. Drying does not have substantial influence on agaritine decomposition; therefore the content of agaritine in dried mushrooms is relatively high (up to 4600 mg/kg). A decrease of agaritine content of up to about 75% occurs during cooking (about 50% of the loss is due to leaching into water). Approximately 20–50% of agaritine remained after 120 min of heating mushroom extracts at 120 °C. Canned mushrooms contain 15–18 mg/kg of agaritine, which corresponds to about 5–8% of the original amount. Agaritine was successfully removed from mushroom water extracts by ethanol fractionation.

Biological effects

Muscarine is an agonist of cholinergic neurons, where acetylcholine acts as a neurotransmitter. Muscarine poisoning, which is not usually regarded as deadly, is manifested about 15–30 min after ingestion of mushrooms by typical symptoms such as sweating and salivation, accompanied by gagging, heavy breathing, decrease in heart rate and blood pressure.

Orellanine and orellinine are nephrotoxins, but the related orelline is a non-toxic compound. Manifestations of poisoning consist of renal impairment after a latency of 2–17 days. The most important symptoms are fatigue, loss of appetite, nausea or even vomiting, headache, severe thirst, urge to urinate, diarrhea, fever, chills without fever and pain in the limbs and the muscles generally. Progressive kidney damage later leads, conversely, to limited production of urine and constipation. Poisoning often results in death or permanent kidney damage, requiring regular dialysis or kidney transplantation.

Most of the approximately 200 species of mushrooms containing psilocybin fall in the genus *Psilocybe*. Psilocybin and psilocin act as hallucinogens, their activity is similar to the activity of the known hallucinogen lysergic acid diethylamide (LSD) as they also affect receptors of serotonine (5-hydroxytryptamine) in the central nervous system. For this reason, many countries have some level of regulation or prohibition of the so-called magic mushrooms.

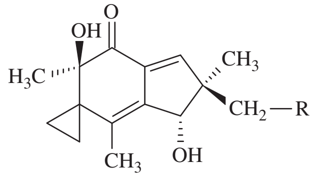
The consumption of false morel is widespread in Europe, but poisoning from consumption of this fungus appears only sporadically, because it can only be caused when raw mushrooms are eaten. Symptoms of gyromitrin poisoning are similar to the symptoms of toadstool poisoning (vomiting, diarrhea, cramps and other symptoms) and ends in 20–30% of cases in coma. Frequent consumption of false morel can generally cause jaundice and neurological disorders. The gyromitrin degradation products (*N*-formyl-*N*-methylhydrazine and *N*-methylhydrazine) are hepatotoxic and carcinogenic substances.

The metabolic fate of agaritine has been linked with the carcinogenity of the mushroom. Hydrazines arising from agaritine, such as 4-(carboxy)phenylhydrazine, β-*N*-[γ-L-glutamyl]-4-(carboxy)phenylhydrazine, 4-(hydroxymethyl)phenylhydrazine and β-*N*-[γ-L-glutamyl]-4-(formyl)phenylhydrazine, and 4-(hydroxymethyl)benzenediazonium salts, could pose some risk as carcinogens. The risk arising from the consumption of mushrooms, with regard to the amount consumed and content of agaritine and its derivatives, requires more detailed evaluation.

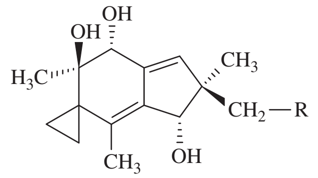
10.3.3.11.5 Terpenoids and other compounds

Structure, nomenclature and occurrence

In addition to toxic nitrogen compounds, fungi also contain many toxic terpenoids. The Jack o'-lantern mushroom (*Omphalotus olearius*, syn. *Clitocybe illudens*, Marasmiaceae) and some other fungi, contains toxic sesquiterpenoids illudins M and S (**10-229**), which are accompanied by non-toxic dihydroilludins M and S (**10-230**). The similarly poisonous Japanese mushroom known as *Tsukiyotake* (*Omphalotus japonicus* syn. *Pleurotus japonicus*) contains 6-deoxyilludins M and S (**10-231**). A compound of similar structure is leaianafulvene (**10-232**), which is found in *Mycena laevigata* (Mycenaceae).

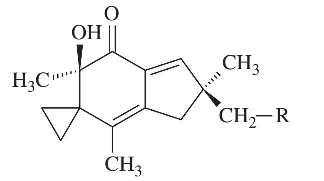


10-229, illudin M, R = H
illudin S (lampterol), R = OH

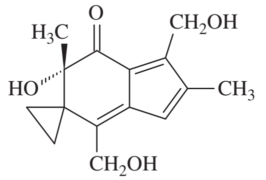


10-230, dihydroilludin M, R = H
dihydroilludin S, R = OH

Lanostane triterpenoid alcohols fasciculols A to F (**10-233**) are examples of biologically active components of the mushroom called sulfur tuft (*Naematoloma fasciculare*, Strophariaceae) and related species. Fasciculols B, C and F and three fasciculol esters, fasciculic acids A, B and C (**10-233**), have calmodulin inhibitory activity and fasciculols E and F are the toxic principles of this mushroom.

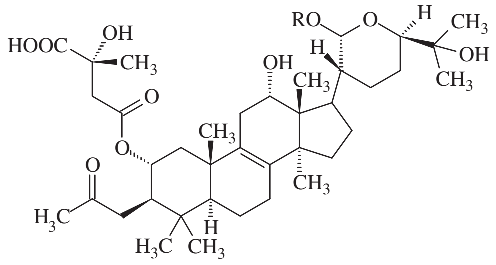


10-231, 6-deoxyilludin M, R = H
6-deoxyilludin S, R = OH



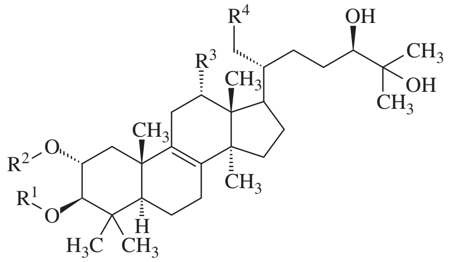
10-232, leaianafulvene

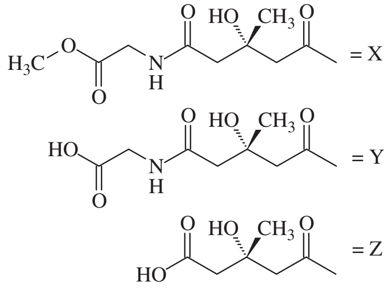
Calmodulin is a calcium-binding messenger protein of eukaryotic cells that mediates many crucial processes, such as inflammation, metabolism, smooth muscle contraction, intracellular movement, short-term and long-term memory and immune response. Toxic substances related to fasciculols and referred to as HS-A, HS-B (**10-234**) and HS-C (**10-235**) are toxins of *Hebeloma spoliatum*, a species of mushroom of the Hymenogastraceae family.



10-234, HS-A, R = H
HS-B, R = OCOCH$_3$

Hebevinosides are toxic triterpenoid diglycosides and monoglycosides. The aglycone structure is similar to the structure of related bitter cucurbitacins that occur in cucurbit plants (Cucurbitaceae, see Section 8.3.4.1.2). Hebevinosides are found in the mushroom *Hebeloma vinosophylum* (Cortinariaceae) growing on high nitrogen media. Approximately 14 different compounds have been identified with different substitutions: $R^1 = H$ or CH_3 and R_2 to $R_3 = H$ or $C(=O)CH_3$. Only glycosides containing D-glucose linked at C-16 are toxic. Examples of hebevinosides are toxic C-3/C-16 diglycosides (**10-236**) and C-16 glycosides (**10-237**).

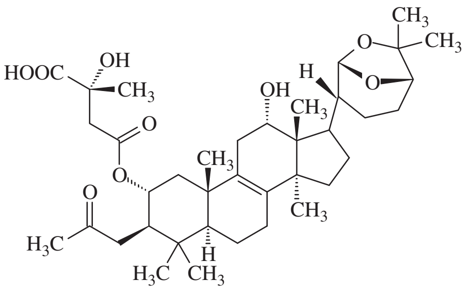




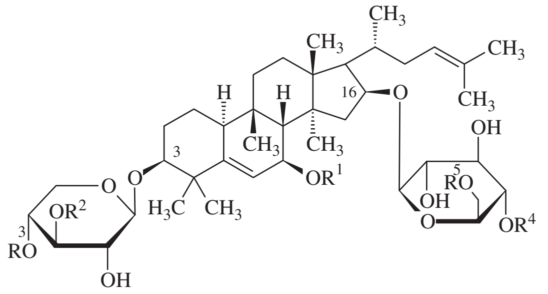
10-233, fasciculols and fasciculic acids

fasciculol A, $R^1 = H$, $R^2 = H$, $R^3 = H$, $R^4 = H$
fasciculol B, $R^1 = H$, $R^2 = H$, $R^3 = OH$, $R^4 = H$
fasciculol C, $R^1 = H$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$
fasciculol D, $R^1 = H$, $R^2 = X$, $R^3 = OH$, $R^4 = H$
fasciculol E, $R^1 = X$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$
fasciculol F, $R^1 = H$, $R^2 = X$, $R^3 = OH$, $R^4 = OH$

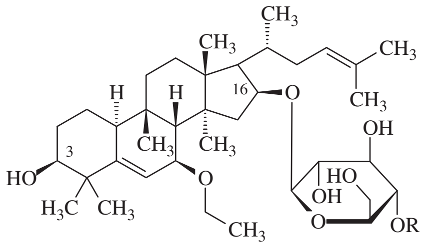
fasciculic acid A, $R^1 = H$, $R^2 = Z$, $R^3 = H$, $R^4 = H$
fasciculic acid B, $R^1 = H$, $R^2 = Z$, $R^3 = OH$, $R^4 = H$
fasciculic acid C, $R^1 = Y$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$



10-235, HS-C

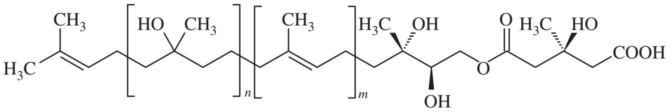


10-236, basic structure of hebevinosides (diglycosides)

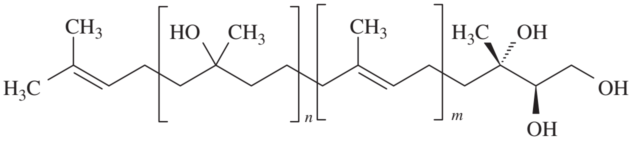


10-237, basic structure of hebevinosides (monoglycosides)

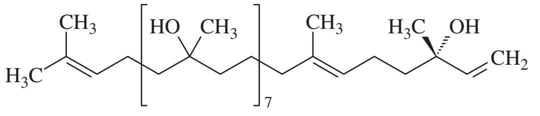
The linear terpenoid compound gymnopilin (**10-238**) is found in the hallucinogenic mushroom *Gymnopilus junonius* (syn. *G. spectabilis*, Cortinariaceae), called big laughing gym, as a mixture of congeners with different numbers of isoprene units ($m = 1$–3, $n = 5$–7). Compounds with more than one double bond ($m > 1$) are toxic. Toxicity depends also on the number of segments (n) with hydroxyl groups. Gymnopilin is furthermore responsible for the bitter taste of mushrooms. Its precursors are gymnoprenol (**10-239**) and gymnopilene (**10-240**).



10-238, gymnopilin

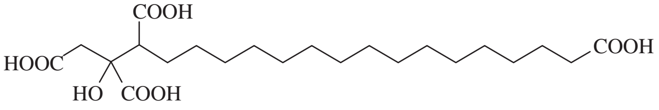


10-239, gymnoprenol



10-240, gymnopilene

The mushroom known as agaricone, quinine conk or white agaric mushroom (*Laricifomes officinalis*, Fomitopsidaceae) contains high amounts (14–16% of dry matter) of the bitter tasting citric acid derivative with a lipophilic substituent on C-2 (derived from palmitic acid), which is known as α-hexadecylcitric acid, α-cetylcitric, agaricinic, agaric or laricic acid, **10-241**). In the past, the mushroom was used for medical purposes and dry sponge impregnated with potassium nitrate was used as tinder.



10-241, agaricinic acid

Reactions and changes

Toxic terpenoids are stable, and fungal foods do not lose their toxicity even during cooking. Insignificant losses of these lipophilic toxins also occur by leaching.

Biological effects

Poisonings caused by illudin M and S, not regarded as deadly, are manifested by typical digestive problems, vomiting and diarrhea. Illudin S and 6-deoxyilludins M and S show some anticarcinogenic activity. Fasciculols E and F cause vomiting, diarrhea and in some cases, convulsions and paralysis. Poisoning may lead to death. Toxins HS-A, HS-B and HS-C can cause paralysis of limbs at higher concentrations (around 100 mg/kg) and diarrhea at lower concentrations (about 45 mg/kg). These substances not only affect the central nervous system, but also the autonomic nervous system. Hebevinosides act as paralytic toxins. Gymnopilin is a neurotoxic hallucinogenic substance. Owing to adverse biological effects (it acts as an antiperspirant, a substance preventing cutaneous respiration), the EU legislation allows agaric acid, as a bitter tasting flavouring, only for alcoholic beverages.

10.3.3.12 Marine toxins

The meat of some marine fish, shellfish and many other animals can be toxic. In most cases, the toxic substance is present throughout the organism, as it is usually caused by the consumption of phytoplankton, microscopic algae (Dinoflagellates), blue–green algae (Cyanobacteria) and diatoms (Diatomeae), which contain various hepatotoxins, neurotoxins, dermatotoxins or intestinal toxins. The primary sources of toxins are bacteria that live with these algae in symbiosis or as epiphytes. A number of toxins are also produced by

some fish and other marine animals. The most important seafood toxins include:

- saxitoxin and its derivatives
- okadaic acid and its analogues (dinophysistoxins)
- azaspiracids
- domoic acid and its analogues
- brevetoxins
- tetrodotoxin and its analogues
- ciguatoxins
- palytoxin.

10.3.3.12.1 Bivalve mollusc toxins

The class Bivalvia (bivalves) of the phyllum Mollusca (molluscs) includes molluscs known as Pacific oysters (*Crassostrea gigas*, Ostreidae), three closely related taxa of blue mussels (*Mytilus edulis*, Mytilidae), hard (round) clams (*Mercenaria mercenaria*, Veneridae) and common whelks (*Buccinum undatum*, Buccinidae), plus many others. The number of cases of serious poisoning by oysters, mussels, clams and other bivalves has been increasing in recent years. Shellfish (a culinary term for exoskeleton-bearing aquatic invertebrates used as food, including molluscs and crustaceans) take in toxic algae from the water as food, and these contain toxins. With the growing proliferation of toxic algae in the oceans, due to eutrophication and climate change, the degree and extent of contamination of shellfish has been increasing. Global aquaculture development, along with the growing popularity of seafood and other factors, is contributing to the increase in health problems after consuming these marine animals. Improved diagnosis of poisoning and closer international monitoring will certainly play a role.

Toxins of shellfish are characterised by strong, sometimes fatal, acute human toxicity. They are thermostable and show no sensory warning signals. Occurrence of toxins in various areas is sporadic, and probably dependent on meteorological and climatic conditions (such as wind direction and speed, water temperature, depth and currents and microlocation) that control the spread of toxic algae. This leads to the utter unpredictability of the degree of contamination and expansion of shellfish, with a consequent high demand for continuous monitoring and control of production sites.

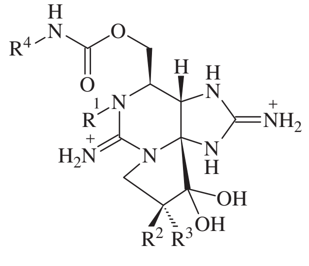
The most important groups of biologically active compounds in shellfish are distinguished by the type of toxic effects (Table 10.26). Some marine toxins are found in the South Seas, while others predominate in colder waters. In Europe, the two most common syndromes of shellfish poisoning are paralytic shellfish poisoning (PSP) and diarrheic shellfish poisoning (DSP). Other types of shellfish poisoning are azaspiracid poisoning (AZP), amnaesic shellfish poisoning (ASP) and neurotoxic shellfish poisoning (NSP).

Paralytic neurotoxins

Structure, nomenclature and occurrence The cause of paralytic shellfish poisoning is the consumption of shellfish contaminated by hydrophilic heterocyclic guanidines, a group of more than 20 related carbamate alkaloid neurotoxins, which are either non-sulfated (saxitoxins; STX), singly sulfated (gonyautoxins; GTX) or doubly sulfated (*N*-sulfocarbamoyl-11-hydroxysulfate toxins; C-toxins). Saxitoxin (**10-242**), is accompanied by structurally related toxins neosaxitoxin and gonyautoxins I to IV, the corresponding *N*-sulfocarbamoyl derivatives ($R^4 = O{-}CO{-}NH{-}SO_3^-$) and decarbamoyl derivatives ($R^4 = OH$). These toxins accumulate in shellfish through ingestion of the dinoflagellates during blooms. The most important sources are dinoflagellates of genera *Gymnodium* (in Europe, Japan and North America), *Alexandrium* (such as *A. tamarense* and *A. catenella* in South America) and *Pyrodinium* (in the Indo-Pacific region). Saxitoxins and related analogues are known to inhibit nerve-muscle transmission by blocking the sodium channels in the excitable membrane and cause a lethal toxicity through breathing muscle paralysis.

Table 10.26 Overview of toxins in molluscs.

Syndrome	Toxin	Symptoms	Occurrece	Limit (EU)
PSP	Saxitoxins	Neurological (mortality)	Europe, Japan, America, Pacific ocean	800 μg/kg
DSP	Okadaic acid and dinophysis-toxins	Gastrointestinal	Europe (northwest), South America	160 μg/kg
AZP	Azaspiracids and analogues	Gastrointestinal	Ireland (Europe)	160 μg/kg
ASP	Domoic acid	Gastrointestinal/short-term memory loss (mortality)	Global (Canada)	20 mg/kg
NSP	Brevetoxins	Neurological (Gastrointestinal)	Florida, Caribean region, New Zealand	



10-242 saxitoxin and its derivatives

saxitoxin, $R^1 = R^2 = R^3 = H$, $R^4 = OCONH_2$
neosaxitoxin, $R^1 = OH$, $R^2 = R^3 = H$, $R^4 = OCONH_2$
decarbamoylsaxitoxin, $R^1 = R^2 = R^3 = H$, $R^4 = OH$
gonyautoxin-1, $R^1 = OH$, $R^2 = H$, $R^3 = OSO_3^-$, $R^4 = OCONH_2$
gonyautoxin-2, $R^1 = R^2 = H$, $R^3 = OSO_3^-$, $R^4 = OCONH_2$
gonyautoxin-3, $R^1 = H$, $R^2 = OSO_3^-$, $R^3 = H$, $R^4 = OCONH_2$
gonyautoxin-4, $R^1 = H$, $R^2 = OSO_3^-$, $R^3 = OH$, $R^4 = OCONH_2$
gonyautoxin-5, $R^1 = R^2 = R^3 = H$, $R^4 = OCONHSO_3^-$
gonyautoxin-6, $R^1 = oH$, $R^2 = R^3 = H$, $R^4 = OCONHSO_3^-$
C1, $R^1 = R^2 = H$, $R^3 = OSO_3^-$, $R^4 = OCONHSO_3^-$
C2, $R^1 = H$, $R^2 = OSO_3^-$, $R^3 = H$, $R^4 = OCONHSO_3^-$
C3, $R^1 = OH$, $R^2 = H$, $R^3 = OSO_3^-$, $R^4 = OCONHSO_3^-$
C4, $R^1 = OH$, $R^2 = OSO_3^-$, $R^3 = H$, $R^4 = OCONHSO_3^-$

Saxitoxin may be found in oysters, common whelks and other species of marine molluscs, but can occur also in some microscopic freshwater blue–green algae (*Aphanizomenon flos-aquae*) and red macroscopic algae of the genus *Jania*, which become the source of saxitoxin in crab meat.

Reactions and changes The toxins are extremely thermostable and cannot be removed by cooking or meat pickling.

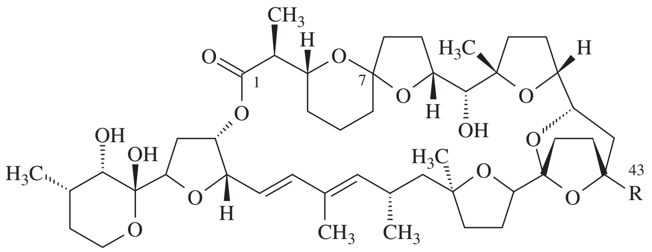
Biological effects Saxitoxin and its derivatives are neurotoxins. The mechanism of action is based on the blockade of conductance in sodium tubules of nerve membrane with subsequent blockade of transmission of nerve impulses. Clinical symptoms may appear within 1 hour and are characterised by numbness in the extremities, which spreads to the neck and face, along with symptoms of severe nausea. In severe cases paralysis of respiratory muscles may occur, at high doses within 2 h after exposure. PSP toxins are quickly eliminated from the body and survival for 24 h means a good chance of recovery. Treatment must be implemented as soon as possible and is only symptomatic. On a global scale, each year about 2000 cases of poisoning occur with approximately 15% mortality. Total toxicity of saxitonins is calculated using toxicity equivalents (TEQ, see Section 12.4.1.5.1) based on the relative toxicities of individual congeners (toxicity equivalency factors, TEF) in the edible portion of molluscs. The effective dose in humans is estimated at 2–30 μg of TEQ per kg body weight, severe poisonings were observed at a dose of 10–300 mg TEQ per kg body weight. Poisoning periodically appears in various localities.

Detection and control The hygienic limit in the EU is currently 80 μg of paralytic toxins in 100 g of edible shellfish portion. Both the EU and other countries have drawn up detailed legislative regulations for the control of saxitoxin complexes.

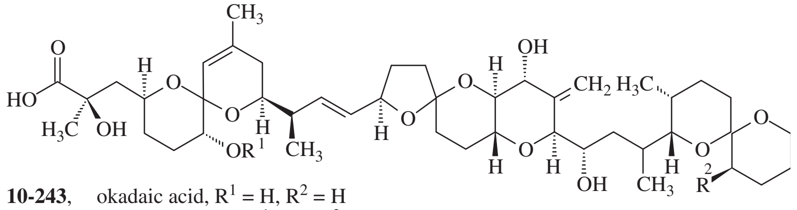
Diarrhoetic toxins

Structure, nomenclature and occurrence Diarrhoetic shellfish poisoning is associated with the consumption of marine shellfish (scallops, oysters and other shellfish) accumulating toxins from dinoflagellates, such as *Dinophysis* and *Prorocentrum* spp. Poisonings of this type are widespread throughout the world, mainly in Japan, Scandinavia, North-West Europe and South America. The cause of the DSP is the okadaic acid group of lipophilic toxins, which include okadaic acid (**10-243**) and its analogues (dinophysistoxins), such as dinophysistoxin-1, known as (*R*)-35-methylokadaic acid, dinophysistoxin-2 (distinct from okadaic acid by the position of the methyl group), which are the main toxic components, and a number of substituted congeners occurring as diol esters and sulfated diol esters in algae and as fatty acid esters in shellfish, such as dinophysistoxin-3, also known as 31-demethyl-35-methylokadaic acid.

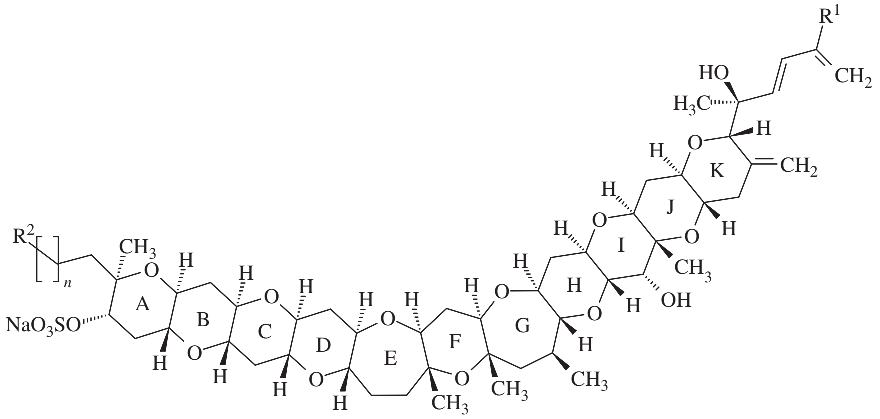
A poisoning with similar symptoms as those caused by okadaic acid can be a result of other related toxins, such as pectenotoxins (PTX, **10-244**) from marine dinoflagellates of the genus *Dinophysis*



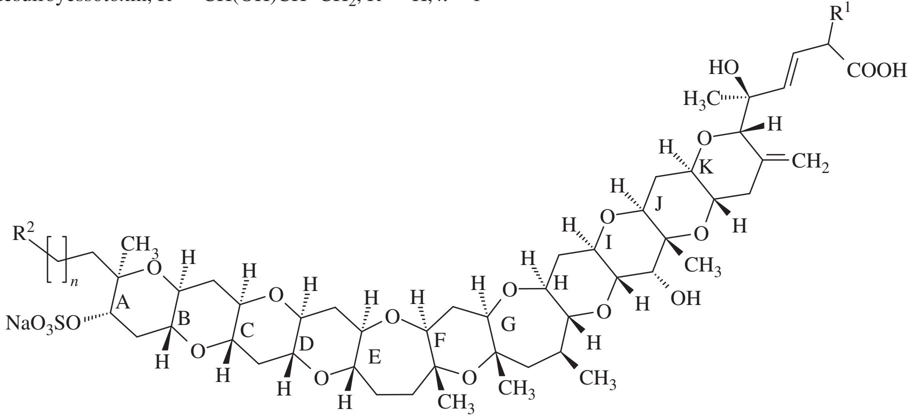
10-244, pectenotoxin-1, R = CH_2OH
pectenotoxin-2, R = CH_3
pectenotoxin-3, R = CH=O
pectenotoxin-6, R = COOH



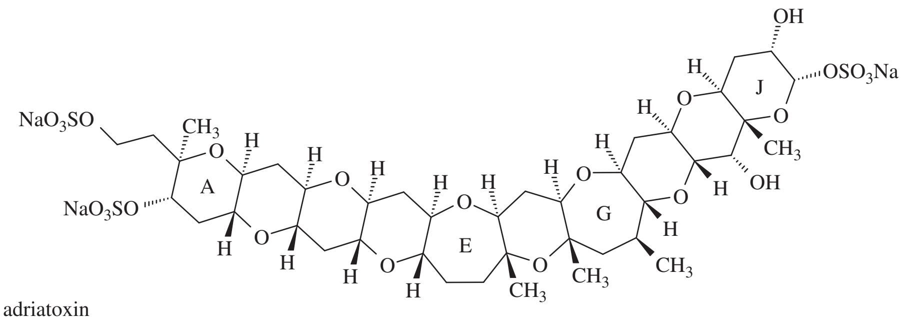
10-243, okadaic acid, $R^1 = H$, $R^2 = H$
dinofysistoxin-1, $R^1 = H$, $R^2 = CH_3$
dinofysistoxin-3, R^1 = zbytek mastné kyseliny, $R^2 = CH_3$



yessotoxin, R^1 =$CH_2CH{=}CH_2$, R^2 = NaO_3SO, *n* = 1
45-hydroxyyessotoxin, R^1 = $CH(OH)CH{=}CH_2$, R^2 = NaO_3SO, *n* = 1
45,46,47-trinoryessotoxin, R^1 =H, R^2 = NaO_3SO, *n* = 1
homoyessotoxin, R^1 = $CH{=}CH_2$, R^2 = NaO_3SO, *n* = 2
45-hydroxyhomoyessotoxin, R^1 = $CH(OH)CH{=}CH_2$, R^2 = NaO_3SO, *n* = 2
1-desulfoyessotoxin, R^1 = $CH(OH)CH{=}CH_2$, R^2 = H, *n* = 1



carboxyyessotoxin, R^1 = $CH_2CH{=}CH_2$, R^2 = NaO_3SO, *n* = 1
carboxyhomoyessotoxin, R^1 = $CH_2CH{=}CH_2$, R^2 = NaO_3SO, *n* = 2



adriatoxin

10-245, yessotoxin and related toxins

and disulfates of cyclic polyethers yessotoxins (YTX, **10-245**), which are produced by planktonic algae *Protoceratium reticulatum* and possibly also by dinoflagellates *Lingulodinium polyedrum*. These toxins have a different mechanism of action and are not included in the hygienic limits for dinophysistoxins.

The most commonly found pectenotoxin in algae is pectenotoxin-2. Pectenotoxin-2 is enzymatically hydrolysed into a pectenotoxin-2 seco acid form by many shellfish species, a reaction that constitutes a detoxification mechanism. Spontaneous epimerisation at C-7 of pectenotoxin-2 seco acid produces

7-epi-pectenotoxin-2 seco acid. Both compounds occur in blue mussels (*Mytilus edulis*) from Ireland as fatty acid esters. Other enzyme-mediated conversions of pectenotoxin-2 include oxidation at C-43 in the scallop *P. yessoensis* to yield pectenotoxin-1, pectenotoxin-3 and pectenotoxin-6 (**10-244**).

The structures of the yessotoxins differ from those of toxins in the diarrhetic shellfish poisoning toxins group and the pectenotoxins but are similar to the brevetoxins and ciguatoxins in having a ladder-shaped polycyclic ether skeleton. Yessotoxin (**10-245**) was evidenced in a marine bivalve mollusc called yesso scallop (*Patinopecten yessoensis*, Pectinidae), but has since been found in a wide range of shellfish from around the world. More recently, 45-hydroxyyessotoxin and 45,46,47-trinoryessotoxin were also isolated from scallops. 45-Hydroxyyessotoxin was isolated from mussels, as were homoyessotoxin, 45-hydroxyhomoyessotoxin, 1-desulfoyessotoxin, adriatoxin, carboxyyessotoxin, carboxyhomoyessotoxin and numerous minor related compounds.

Reactions and changes Toxins are thermostable, as well as other toxins in shellfish and structurally similar ciguatera toxins of fish, and cannot be removed by heat treatments.

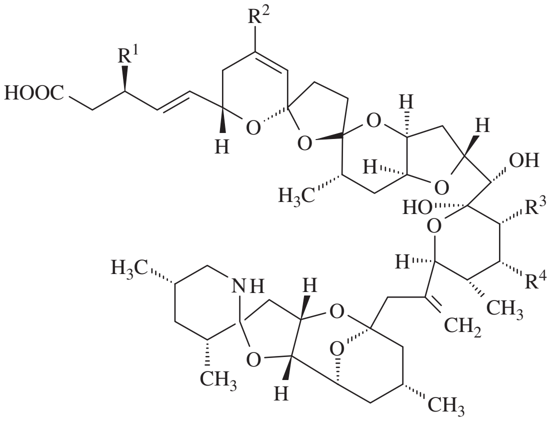
Biological effects Okadaic acid is an inhibitor of phosphatases, which leads to phosphorylation of proteins controlling excretion of fluids from the intestine. The main symptoms of poisoning are indigestion exhibited by stomach pain, nausea, vomiting and diarrhea. These symptoms occur quickly (30 min after ingestion of shellfish) and disappear spontaneously after 3–4 days without the need for hospitalisation. Data on long-term toxicity of dinophysistoxins in humans are lacking. In laboratory rodents, high doses of these toxins act as carcinogenicity promoters, but their genotoxicity was not confirmed.

The existing hygienic limits for diarrhoetic toxins in the EU are based on acute toxicity in the form of ARfD (Acute Reference Dose). Toxicity of the whole group of toxins is translated into okadaic acid using toxic equivalency factors: TEF for okadaic acid and dinophysistoxin-1 = 1, dinophysistoxin-3 = 2; TEF for dinophysistoxin-3 is equal to the corresponding non-esterified compounds (okadaic acid, dinophysistoxin-1 and dinophysistoxin-2). Cases of human poisoning have been observed in areas having 0.8 mg okadaic acid toxicity equivalents per kg body weight.

Detection and control In the EU the currently applied hygienic limit is 160 μg of okadaic acid toxicity equivalents per kg for edible molluscs, but this limit is being discussed in terms of adequate protection during the eating of large portions of this food.

Azaspiracids

Structure, nomenclature and occurrence The group of marine toxins known as azaspiracids (AZA) was only recently discovered to be the cause of shellfish poisoning in humans. Their source is the algae of the genus *Protoperidinium*. Azaspiracids are a group of polyethers (such as okadaic acid), but they contain a nitrogen heterocyclic amine piperidine (**10-246**). About 20 different derivatives have been identified, of which azaspiracid-1 (AZA1) and azaspiracid-3 (AZA3) are relevant to the toxicity.



10-246, azaspiracids

AZA1, $R^1 = R^2 = H$, $R^3 = CH_3$, $R^4 = H$

AZA2, $R^1 = H$, $R^2 = CH_3$, $R^3 = CH_3$, $R^4 = H$

AZA3, $R^1 = R^2 = R^3 = R^4 = H$

AZA4, $R^1 = OH$, $R^2 = R^3 = R^4 = H$

AZA5, $R^1 = R^2 = R^3 = H$, $R^4 = OH$

Reactions and changes Azaspiracids are thermostable and cannot be removed by heat treatment.

Biological effects AZP syndrome was first described in 1995 after a poisoning case involving the eating of blue mussels (*Mytilus edulis*) from Ireland. In subsequent years, poisoning cases have been reported from other European countries, but the mussels were always of Irish origin. Poisoning is characterised by digestive problems: nausea, vomiting, diarrhea and stomach pain. In all cases there is spontaneous recovery within a few days.

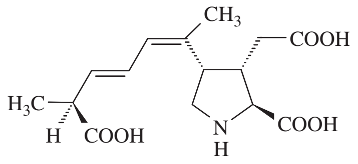
As in other toxins, the hygienic limits are based on the acute toxicity (ARfD) and the total exposure to individual azaspiracid analogues is converted into AZA1 using TEF equivalents. The lowest dose that causes toxic effects is estimated to be approximately 2 mg of AZA1 equivalents per adult person.

Detection and control The EU hygienic limit is (similar to the case of DSP) 160 μg of AZA1 toxicity equivalents per kg of edible shellfish portion. After the introduction of mandatory monitoring, low levels of toxins in shellfish were registered in different EU countries. In one case (France in 2008), there was a mass poisoning (of 208 people). In this case it was shown that the hygienic limit had been exceeded by several times.

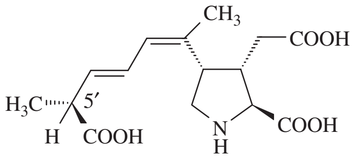
Amnestic toxins

Structure, nomenclature and occurrence ASP is a less common type of poisoning. It occurs after ingestion of shellfish contaminated by a specific toxic amino acid referred to as domoic acid (**10-247**), a naturally occurring neuroexcitatory toxin produced primarily by the marine diatom *Pseudonitzschia multiseries*, which is widespread

in warm and in colder seas. Other compounds of similar structure include domoic acid diastereomer 5′-domoic acid (**10-248**) and ten domoic acid isomers (isodomoic acids A to H, **10-249**). The first human poisoning case was reported in 1987 by ingestion of domoic acid-contaminated blue mussels (*Mytilus edulis*). Contamination was later found in various molluscs. Domoic acid was originally isolated in Japan from red algae *Chondria armata* called *doumoi*.



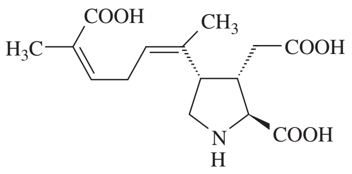
10-248, 5′-domoic acid



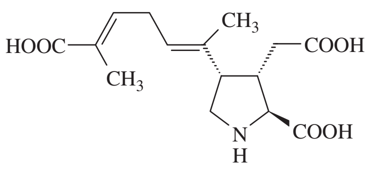
10-247, domic acid

Reactions and changes Domoic acid is thermostable and cannot be removed by heat treatment. It has been shown that domoic acid may degrade in acidic media, but the practical importance of such treatment (e.g. pickling in vinegar) is not known.

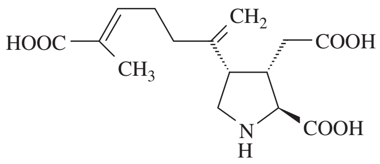
Biological effects Amnaesic shellfish poisoning is characterised by relatively mild gastrointestinal symptoms (vomiting, stomach pain and diarrhea). In serious cases, especially in the elderly (over 50 years) and also in impaired and sick individuals, the neurotoxic symptoms accompanied by persistent amnaesia may outweigh the gastrointestinal symptoms. A case of public poisoning was reported



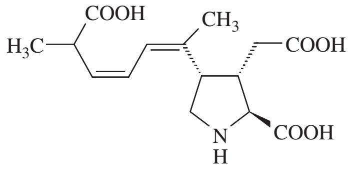
isodomoic acid A



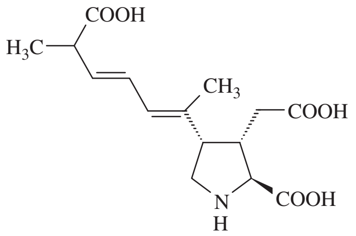
isodomoic acid B



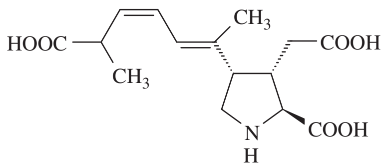
isodomoic acid C



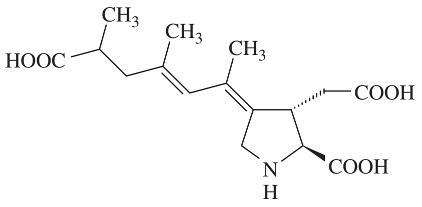
isodomoic acid D



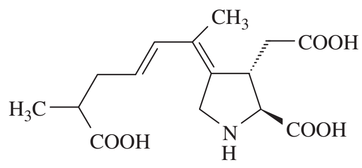
isodomoic acid E



isodomoic acid F



isodomoic acid G



isodomoic acid H

10-249, isodomoic acids

in 1987 in Canada (over 100 people, resulting in four deaths) after ingestion of blue mussels (*Mytilus edulis*). The mechanism of toxic effect is the interaction of toxins with specific glutamate receptors in the brain. Continuous stimulation of receptors leads to the accumulation of Ca^{2+} in nerve cells with consequent damage of the nervous tissue. Mild gastrointestinal symptoms of poisoning in humans were found after the intake of domoic acid at a concentration of 0.9–1.9 mg per kg body weight. Severe neurological changes start at domoic acid levels ranging from 1.9 to 4.2 mg per kg of body weight.

Detection and control In the Canadian study, the lowest concentration of domoic acid in shellfish leading to mild gastrointestinal problems was estimated at 200 mg/kg of edible portion, and the hygienic limit was set at 20 mg/kg of edible portion. This limit is also applied in other countries, including the EU. Contamination of shellfish is occasionally detected in shellfish in different countries, but since the introduction of the limit no cases of poisoning have occurred.

Neurotoxic toxins

Structure, nomenclature and occurrence Neurotoxic brevetoxins are metabolites that are found in the microscopic algae *Gymnodinium breve* (syn. *Ptychodiscus brevis*), which occur in the Gulf of Mexico, the Caribbean region and New Zealand. The extreme algae blooms, for example, along the coast of Florida, creates a so-called red tide, which is a frequent cause of mass fish poisoning. Toxins present in the air as an aerosol may cause temporary inhalation problems when inhaled.

In humans, these neurotoxins cause irritation of the eyes and occasionally poisoning when consuming different bivalves that eat these algae. The most effective toxin produced by algae *Gymnodinium breve* is brevetoxin A (**10-250**), which occurs together with brevetoxin B (**10-251**).

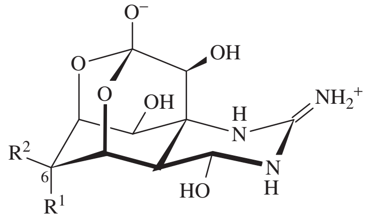
Reactions and changes Brevetoxins, as well as structurally similar ciguatoxins, are thermostable and cannot be removed by heat treatments.

Biological effects Symptoms of poisoning by brevetoxins are similar to symptoms of ciguatera fish poisoning (CFP). These neurological symptoms accompanied by digestive difficulties disappear spontaneously within 48 h.

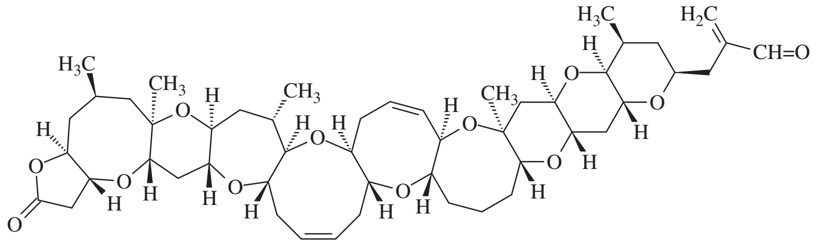
10.3.3.12.2 Fish toxins

Tetrodotoxin

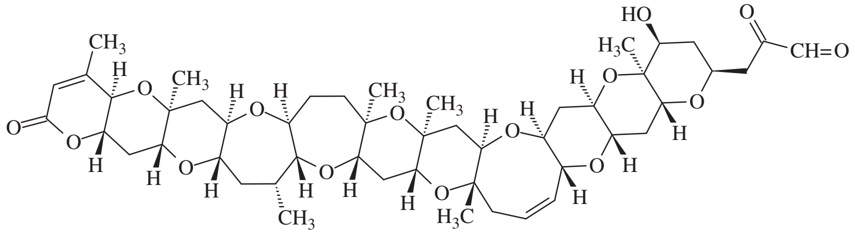
Structure, nomenclature and occurrence The most effective fish toxin is tetrodotoxin (**10-252**), *O*-methyl-*O*′,*O*′′-isopropylidene-tetrodotoxin hydrochloride, which is accompanied by related 6-epitetrodotoxin, 11-deoxytetrodotoxin, 11-oxotetrodotoxin, (*R*)-11-nortetrodotoxin-6-ol, (*S*)-11-nortetrodotoxin-6-ol and



10-252, tetrodotoxin, R^1 = OH, R^2 = CH_2OH
6-epitetrodotoxin, R^1 = CH_2OH, R^2 = OH
11-deoxytetrodotoxin, R^1 = OH, R^2 = CH_3
11-oxotetrodotoxin, R^1 = OH, R^2 = CHO
(*R*)-11-nortetrodotoxin-6-ol, R^1= H, R^2= OH
(*S*)-11-nortetrodotoxin-6-ol, R^1= OH, R^2= H
chiriquitoxin, R^1 = OH, R^2 = CH(OH)CH(NH_2) COOH, (1*R*, 2*S*)-isomer



10-250, brevetoxin A



10-251, brevetoxin B

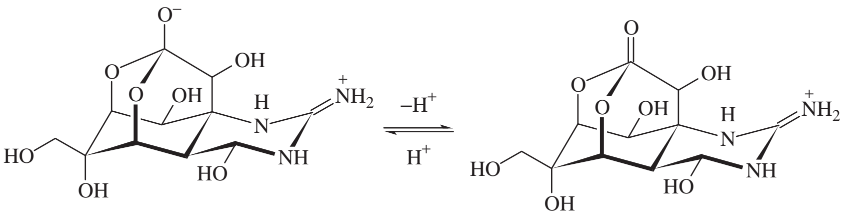


Figure 10.23 Tetrodotoxin salt tautomers.

chiriquitoxin. Both epimers of 11-nortetrodotoxin are probably decarboxylation products of hypothetical tetrodonic acid. Tautomerism is typical for tetrodotoxin and its derivatives; these substances exhibit some properties of ketones and some properties of alcohols (Figure 10.23).

Tetrodotoxin is found in fish known by various names, such as puffer fish, puffers, balloon fish, blowfish and puffu of the family Tetraodontidae, of the genera *Tetraodon* and *Fugu*, which are consumed in Japan as a delicacy. The toxin is not present in the meat, but is present in the female roe, liver, intestines and skin. The source of the toxin is algae consumed by fish, accordingly, bacteria originally classified in the genus *Pseudomonas* and later to the genus *Alteromonas*. The current name is *Shewanella alga*. Tetrodotoxin and chiriquitoxin are also found in some Central American salamanders and frogs (*Atelops chiriquiensis*).

Reactions and changes Toxicity of fish can be reduced by extraction with water or water acidified with acetic acid. Toxicity of roe can also be reduced by the action of alkaline reagents. During heating, the toxic compounds are partially degraded to form less toxic derivatives. Sometimes even the meat may be toxic due to the migration of the toxin from the skin to the internal tissues.

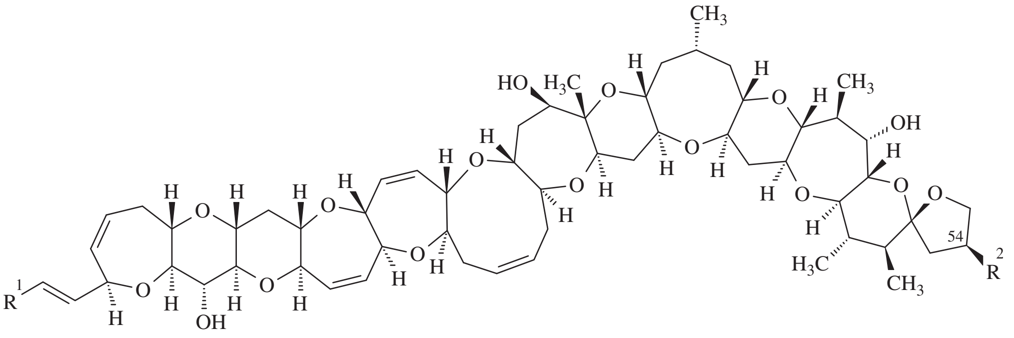
Biological effects Tetrodotoxin is a powerful neurotoxin (paralytic poison, which prevents the function of neuronal channels for Na^+ ions). More than 60% of poisonings are fatal. About as toxic as tetrodotoxin ($LD_{50} = 8–10\ \mu g/kg$ in mice) is chiriquitoxin, while other compounds are less toxic.

Ciguatoxins

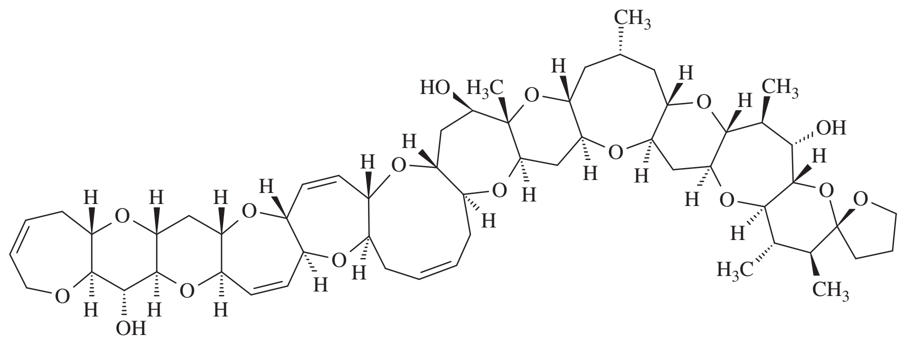
Structure, nomenclature and occurrence Poisoning caused by species of moray eels, such as giant moray (*Gymnothorax javanicus*, Muraenidae), found at coral reefs in tropical seas in the Indo-Pacific, is known as ciguatera fish poisoning (CFP). Mainly responsible for the poisoning are ciguatoxin and maitotoxin and its congeners, which are members of the polycyclic ether family of marine toxins found in the fish flesh contaminated with toxins. Ciguatoxin (**10-253**) and its polar congeners arise by the action of fish oxidases from polar congeners derived from dinoflagellates (organisms of the phylum Dinoflagellata). Dinoflagellates adhere to coral, algae and seaweed, where they are eaten by herbivorous fish that in turn are eaten by carnivorous fish, such as giant moray. For example, ciguatoxin-3C (formerly known as gambiertoxin 4b, **10-254**) is detected in the epiphytic dinoflagellate species *Gambierdiscus toxicus* (Goniodomataceae) along with other related compounds, such as gambierol (**10-255**). The congener 54-deoxyciguatoxin (ciguatera-4B, **10-254**), which is found only in fish, is the immediate precursor of ciguatoxin. A related polycyclic ether is maitotoxin (**10-256**).

Reactions and changes Ciguatoxins do not affect the organoleptic characteristics of meat, are thermostable and cannot be removed by thermal procedures. Long-term water extraction may partially reduce their content.

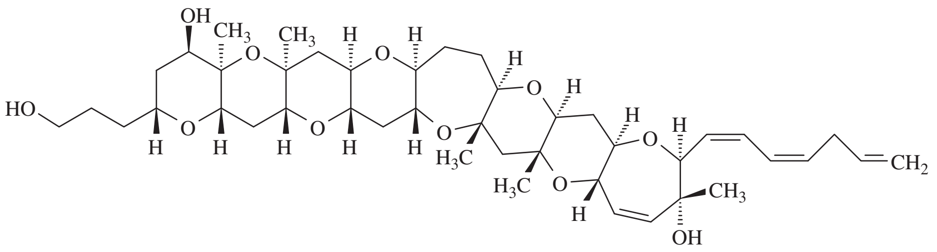
Biological effects Ciguatera fish poisoning is the most common poisoning after eating fish. Symptoms of poisoning appear a few



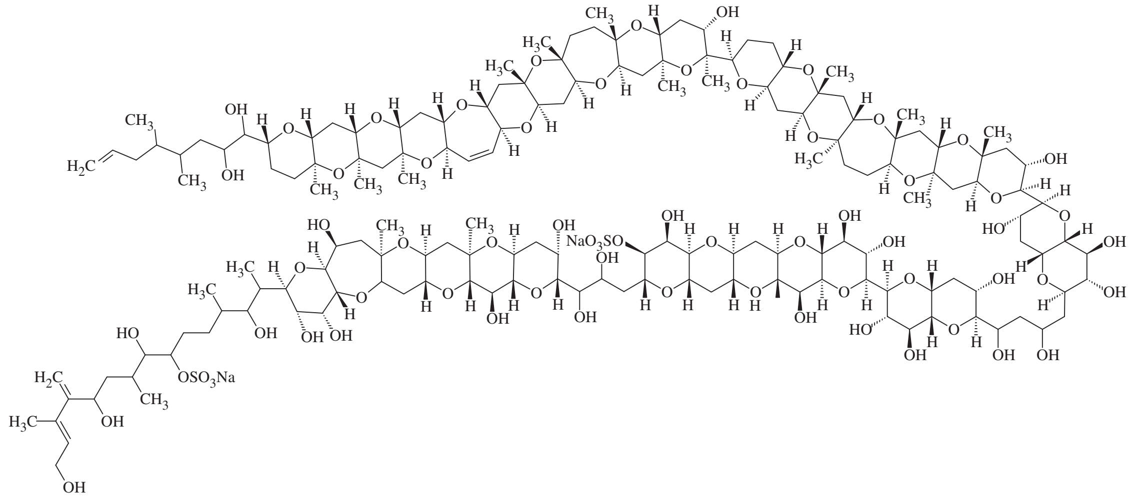
10-253, ciguatoxin, $R^1 = CH(OH)CH_2OH$, $R^2 = OH$
ciguatoxin-4B, $R^1 = CH{=}CH_2$, $R^2 = H$



10-254, ciguatoxin-3C



10-255, gambierol

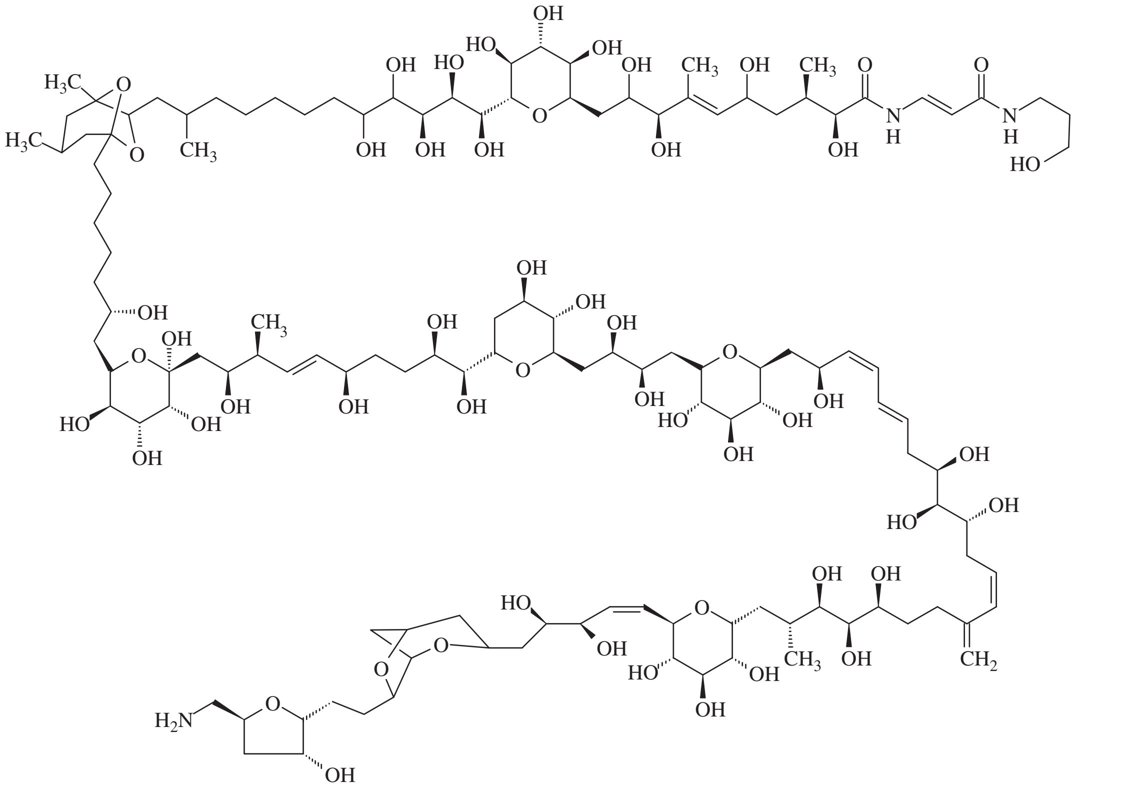


10-256, maitotoxin

hours after eating as digestive problems and subsequent neurological symptoms, muscle pain, weakness, low blood pressure, change in heart rate and more. The variety of symptoms is often associated with the presence of maitotoxin and other bioactive compounds. Mortality caused by this poisoning is low. Ciguatoxin is about ten times more toxic than less polar congeners (such as ciguatoxin-4B). Maitotoxin belongs to the most toxic non-protein compounds (LD_{50} = 50 ng/kg in mice).

Palytoxin

Structure, nomenclature and occurrence Palytoxin is a group of related lipophilic toxins, the structure of which may vary in different species of zoanthids (animals within the order Zoantharia) that are commonly found in coral reefs. Palytoxin (**10-257**) was first isolated from the Hawaiian seaweed-like coral zoanthid *Palythoa toxica*. Later it was found in the red algae *Chondria armata* (Rhodomelaceae), crabs of the genera *Demania* and *Lophozozymus* (Xanthidae), fish species pink tail triggerfish (*Melichthys vidua*, Balistidae) and scrawled filefish (*Aluterus scriptus*, Monacanthidae), and was also identified in the liver of parrot fish (*Ypsiscarus ovifrons*). Even though the appearance of palytoxin was initially restricted to tropical areas, the recent occurrence in micro alga, the dinoflagellate of the genus *Ostreopsis* in Mediterranean Sea, point to a worldwide dissemination probably related to climate change.



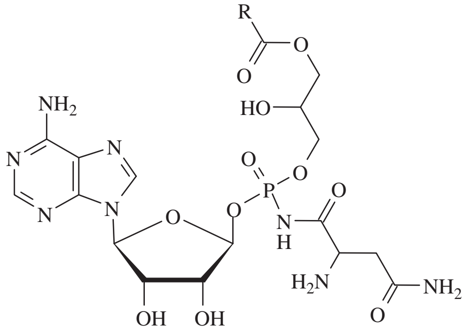
10-257, palytoxin

Reactions and changes Palytoxin group toxins are relatively stable. No data on their changes during cooking or industrial processing are known.

Biological effects Palytoxin targets the sodium–potassium pump protein by binding to the molecule analogously as tetrodotoxin. It probably also has carcinogenic effects. Palytoxin is considered to be one of the most toxic non-peptide substances known, second only to maitotoxin ($LD_{50} = 0.1–0.3\,\mu g$ in mice) of the same class as botulotoxin. Palytoxin is responsible for lethal poisonings caused by eating the meat of crabs *Demania reynaudii* in the Philippines. Poisoning causes similar symptoms as tetrodotoxin poisoning, the symptoms of which are angina-like chest pains, asthma-like breathing difficulties, tachycardia (racing pulse), unstable blood pressure with episodes of low blood pressure and haemolysis. The onset of symptoms is rapid, with death occurring within minutes.

Other toxins

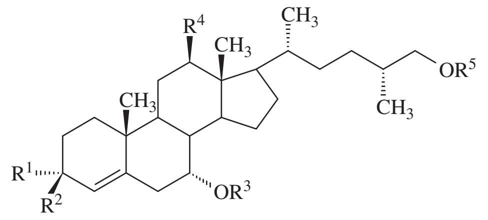
In saltwater fish a number of other biologically active metabolites of algae occur that cause different types of poisoning. In addition to these secondary metabolites, some fish also produce their own toxins. For example, the fish *Stichaeus grigorjewi* (Stichaeidae), common in the Northwest Pacific, contains toxic lipoproteins, which are known as α-, β- a γ-lipostichaerins, in the roes. In addition to neutral lipids, lipostichaerins contain unusual toxic phospholipid dinogunellin, which is composed of adenosine and



10-258, dinogunellin

2-aminosuccinamide (**10-258**, R = fatty acid residue). The same ichthyotoxin was found in the eggs of other fish that are toxic, especially at the time of spawning. Lipoproteins easily denature by heat, but the toxic component dinogunellin is stable. The toxin affects the central nervous system, but poisoning is not usually fatal.

The blood of some fish species of the garden eel family Congridae and the freshwater eel family Anguillidae, for example the blood of European eel (*Anguilla anguilla*), contains ichthyohaematoxin. This protein decomposes with acids, bases and by the action of proteolytic enzymes and is denatured by heating to a temperature of 70 °C or higher or by UV irradiation. Different individuals are sensitive to the eel's blood in different ways. Poisoning occurs rarely



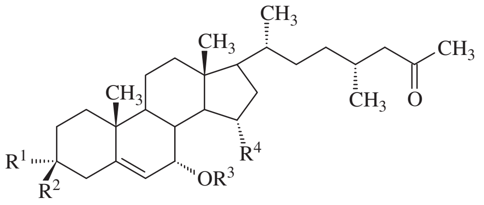
10-259, pavoninin 1, R^1 = OH, R^2 = OH, R^3 = β-D-Glc*p*NAc-(1®, R^4 = H, R^5 = $COCH_3$
pavoninin 2, R^1 = OH, R^2 = OH, R^3 = β-D-Glc*p*NAc-(1®, R^4 = H, R^5 = H
mosesin 1, R^1 = OH, R^2 = H, R^3 = β-D-Gal*p*6Ac-(1®, R^4 = OH, R^5 = $COCH_3$
mosesin 5, R^1 = OH, R^2 = H, R^3 = β-D-Gal*p*-(1®, R^4 = OH, R^5 = $COCH_3$

because the blood is toxic only when consumed in large quantities. Toxicity depends on the season and is the highest at the time of spawning.

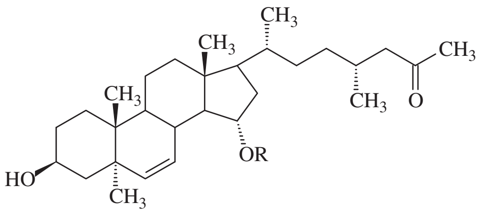
Ichthyocrinotoxins occur in toxic skin secretions of many fish species of the families Soleidae, Ariidae, Ostraciidae, Tetraodontidae, Gobiidae, Serranidae, Batrachoididae and Muraenidae. They show strong haemolytic effects and serve as protective agents against microorganisms and parasites. The structure of toxins is dependent on their origin. Toxic secretion components are peptides, steroids and various fatty acid derivatives that have a bitter taste and the food is unacceptable, but poisoning of humans is unknown.

For example, the skin secretion of the fish peacock sole *Pardachirus pavoninus* (Soleidae), occurring in the Indo-Pacific region, contains linear peptides produced by a series of toxic glands along the bases of dorsal- and anal-fin rays. These toxic peptides, composed of 33 amino (2800 Da), are called pardaxin 1, pardaxin 2 and pardaxin 3. For example, the primary structure of pardaxin 1 is formed by the following sequence of amino acids: Gly-Phe-Phe-Ala-Leu-Ile-Pro-Lys-Ile-Ile-Ser-Ser-Pro-Leu-Phe-Lys-Thr-Leu-Leu-Ser-Ala-Val-Gly-Ser-Ala-Leu-Ser-Ser-Ser-Gly-Glu-Gln-Glu. Other pardaxins have similar structures. As with other polypeptides, paradaxins are labile compounds that are relatively easily hydrolysed by proteolytic enzymes, and partly also by cooking.

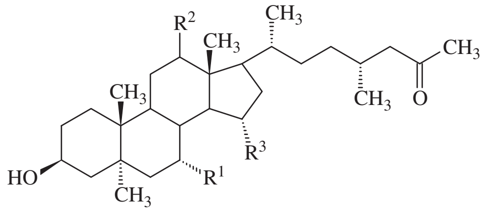
Other compounds present in the toxic secretion of peacock sole are glycosides of steroid toxins called pavoninins (**10-259** to **10-262**). The skin secretion of a related fish species known as finless sole (*P. marmoratus*) are structurally related lipophilic toxins called mosesins (**10-259**, **10-260** and **10-262**).



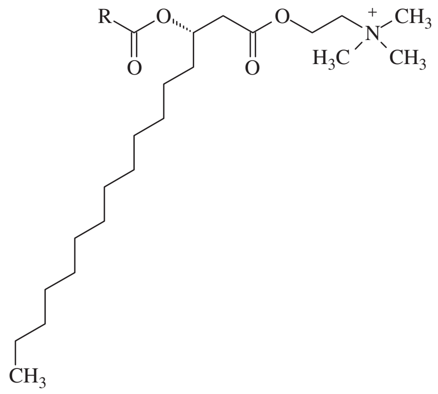
10-260, pavoninin 3, R^1 = OH, R^2 = H, R^3 = H, R^4 = β-D-Glc*p*NAc-(1®
pavoninin 5, R^1 = H, R^2 = OH, R^3 = H, R^4 = β-D-Glc*p*NAc-(1®
mosesin 2, R^1 = H, R^2 = OH, R^3 = β-D-Gal*p*-(1®O, R^4 = H



10-261, pavoninin 6, R = β-D-Glc*p*NAc-(1®



10-262, pavoninin 4, R^1 =, R^2 = H, R^3 = β-D-Glc*p*NAc-(1®O
mosesin 3, R^1 = β-D-Gal*p*6Ac-(1®O, R^2 = OH, R^3 = H
mosesin 4, R^1 = β-D-Gal*p*-(1®O, R^2 = OH, R^3 = H



10-263, pahutoxin, R = CH_3
homopahutoxin, CH_2CH_3

The skin secretion of *Ostracion lentiginosus* and of various other members of the Ostraciidae family contains the toxin pahutoxin, (*S*)-2-(3-acetyloxyhexadecanoyl)oxyethyltrimethylazanium chloride (choline chloride ester of 3-acetylhexadecanoic acid, **10-263**). Pahutoxin is a cationic surfactant, which can be released to the surrounding area, which negatively affects other fish, as the toxic effect is similar to the effect of saponins. The secretion of Hawaiian boxfish *O. immaculatus* contains pahutoxin and related homopahutoxin, (choline chloride ester of 3-propionylhexadecanoic acid), which has the systematic name (*S*)-(2-(3-propanoyloxyhexadecanoyl)oxyethylazanium chloride (**10-263**).

11

Food Additives

11.1 Introduction

In the broadest sense, food additives are substances or mixtures intentionally added to food to improve its quality. Basically, anything that is not naturally a part of a food is considered a food additive. Legally, the term food additive means 'any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its byproducts becoming directly or indirectly a component of such foods' (European Council Directive 89/107/EEC). Food additives have always played a vital role in food supply. Before modern food manufacturing, salting fish and meat was one of the oldest techniques to extend their shelf life. Today's food additives allow the world's growing urban population to have a variety of toxicologically safer and nutritionally and organoleptically more valuable food all the year round, without the inconvenience of daily shopping and cooking.

The use of food additives has a number of advantages, such as:

- reduced product spoilage and extended shelf life of foods using preservatives, which prevent poisonings by bacterial and fungal origin, including life-threatening botulism;

- improved or maintained nutritional value by the addition of vitamins and minerals, which helps to reduce malnutrition;

- prevention of formation of potentially toxic oxidation products of lipids and other food constituents by antioxidants and prevention of formation of various off-flavours and off-tastes, while maintaining palatability and nutritional value, which is associated with the content of easily oxidisable essential fatty acids and lipophilic vitamins;

- giving food a desirable feel by the addition of emulsifiers, thickeners and gelling agents, which allow the product to maintain a consistent texture and wholesomeness, and enable production of low-energy foods and novel foods with reduced sugar and fat content;

- enhancing flavour by the addition of spices, natural and synthetic flavours, or imparting a desired colour by the addition of natural pigments or synthetic colorants, which enhance the appearance of foods to meet consumer expectations.

Of course, some additives could be eliminated if we were willing to grow our own food and spend many hours cooking and canning, or if we accepted increased risks of food spoilage. The beneficial use of additives, however, is offset by some risks. Short-term adverse effects of sulfur dioxide, for example, may manifest in some particularly sensitive individuals by gastric irritation, nausea, diarrhoea, asthma attacks and skin rashes. The negative effect of nitrites depends on their potential induction of methaemoglobinaemia, especially in children (see Section 6.6.1.2.2) and their reaction with secondary amines to form carcinogenic nitrosamines. In test animals, saccharine, which is banned in some countries, interferes with blood coagulation, blood sugar levels and digestive function and may cause cancer of the bladder, uterus, ovaries, skin and blood vessels. However, the risks of the long-term use of additives by humans are not well documented; nevertheless, the use of some additives (such as nitrite and saccharin) is potentially problematic and other substitutes are being sought. Especially important is the continuous evaluation of the potential risks in the light of new knowledge in the field of toxicology.

The EU legislation on food additives is based on the principle that only those additives that are explicitly authorised may be used. Most food additives may only be used in limited quantities in certain foods. If no quantitative limits are anticipated for the use of a food additive, it must be used according to good manufacturing practice, which means only as much as necessary to

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achieve the desired technological effect. Food additives may only be authorised if:

- there is a technological need for their use
- they do not mislead the consumer
- they present no hazard to the health of the consumer.

To assess the possible harmful effects of a food additive, it must be subjected to appropriate toxicological testing and evaluation by the Scientific Committee on Food, an expert panel that advises the European Commission in questions relating to food. The evaluation should also take into account, for example, any cumulative, synergistic or potentiating effect of its use, and the phenomenon of human intolerance to substances foreign to the body. All food additives must be kept under continuous observation and must be re-evaluated in the light of changing conditions of use and new scientific information. The use of food additives must always be labelled on food product packaging by their category (e.g. antioxidant, preservative or colour) with either their name or E-number. E-Numbers are codes for food additives for use within the European Union (the 'E' prefix means for 'Europe') and Switzerland.

The source of international food standards, *Codex Alimentarius*, is published by the Food and Agricultural Organization and World Health Organization (FAO/WHO) of the United Nations. In Europe, the sources of food standards are EU directives. The Codex Committee on Food Additives and Contaminants (CCFAC) deals with the issue of additives, contaminants and natural toxins in food, and also proposes the Maximum Residue Level (MRL, in mg/kg food) for food additives, for example, in the case of arbitration of the World Trade Organisation. The advisory body of CCFAC is the Joint FAO/WHO Expert Committee on Food Additives (JECFA), composed of experts from Member States and Associated Countries of the FAO/WHO. Recommendations for the EU come from a committee of experts nominated by an authority called the Scientific Committee for Food (SCF). These recommendations are converted into legislative form in different countries. In the United States, for example, the source of food standards is the U.S. Food and Drug Administration (FDA).

In this chapter, food additives are classified according to their use as substances that prolong food shelf life, regulate flavour (odour and taste), colour and texture and increase the biological value and technological quality of food. Substances prolonging the shelf life of foods include antimicrobial substances (preservatives) and antioxidants. Substances enhancing odour and taste include food aromas and sweeteners, both natural and synthetic, acidulants and acidity regulators and bitter substances. The section devoted to colours includes natural pigments and synthetic dyes used as food additives, inorganic pigments and bleaching agents (including flour treatment agents). Substances used for regulation of texture are thickeners, gelling agents and emulsifiers. The focus is on vitamins and minerals with substances that increase the biological value of food. Other food additives include humectants, aroma carriers, dispersion stabilisers, anti-caking, bulking, raising, clarifying, haze forming, anti-foaming and glazing agents, lubricants and releasing substances, sequestrants, propellants and packaging gases, catalysts and solvents used in different food technologies. For each of these groups of food additives, the chapter will focus on the relevant legislation and health aspects.

11.2 Substances prolonging the shelf life of foods

There are two basic types of agents that can prolong the shelf life of foods:

- antimicrobial agents, known as preservatives, which are used to protect food against undesirable microorganisms;
- antioxidants, which protect lipids and some other sensitive food constituents (such as vitamins) from oxidation.

11.2.1 Preservatives

Antimicrobial substances (preservatives) prolong shelf life of foods by protecting them against deterioration caused by undesirable microorganisms. Preservation usually involves preventing the growth of bacteria and fungi (yeasts and moulds). A large number of natural components of food materials have antimicrobial effects, and a number of other substances can be intentionally added to foods. Used as preservatives (E-number range is E200–E299) are some organic acids and their salts or inorganic acids, their oxides, salts and esters (E200–E219, E242, E280–E285), including sulfur dioxide and some derived compounds (E220–E228), nitrites and nitrates (E249–E252). Some phenols (E230–E232) and thiabendazole (E233), which is a representative of synthetic fungicides, are active as fungicides. Certain antibiotics (E234 and E235) have special uses, and the enzyme lysozyme is listed with additional chemicals (E1105). Other organic and inorganic substances also show preservation effects, and are formally classified in different groups of food additives (such as acetic acid, ranked among the acidulants) or substances that are not classified as food additives (e.g. sucrose and sodium chloride).

In industrial and also in culinary practice, methods based on physical principles are widely used in addition to these chemical methods of food preservation. Of these, it is primarily food preservation by heat treatment (pasteurisation and sterilisation), cold (chilling and freezing), drying (dehydration), irradiation, and more recently by high pressure, which attract the most attention.

11.2.1.1 Acids and their derivatives

11.2.1.1.1 Benzoic acid

Benzoic acid (E210) and benzoates (E211–E213) are primarily used as antifungal agents. Most fungi (yeasts and moulds) are inhibited by undissociated acid, the concentration of which is 500–1000 mg/kg. Some bacteria are inhibited at a concentration of 100–200 mg/kg, but many bacteria are inhibited by much higher

Table 11.1 Antimicrobial spectrum of benzoic acid.

Microorganisms	pH	Minimum inhibitory amount (mg/l)	Microorganisms	pH	Minimum inhibitory amount (mg/l)
Bacteria			***Yeasts***		
Bacillus cereus	6.3	500	*Hansenula* spp.	4.0	180
Escherichia coli	5.2–5.6	50–120	*Rhodotorula* spp.	–	100–200
Lactobacillus spp.	4.3–6.0	300–1800	*Saccharomyces bayanus*	4.0	330
Lysteria monocytogenes	5.6	2000–3000	*Zygosaccharomyces* spp.	4.8	1000–4800
Micrococcus spp.	5.5–5.6	50–100	***Moulds***		
Pseudomonas spp.	6.0	200–480	*Aspergillus* spp.	3.0–5.5	200 > 4000
Streptococcus spp.	5.2–5.6	200–400	*Bysochlamys nivea*	3.3	500
Yeasts			*Cladosporium herbarum*	5.1	100
Candida crusei	–	300–700	*Mucor racemosus*	5.0	30–120
Debaromyces hansenii	4.8	500	*Penicillium* spp.	2.6–5.0	30–2000
Pichia membranefaciens	–	700	*Rhizopus nigricans*	5.0	30–120

concentrations (Table 11.1). According to the type of food, the concentration used ranges from 150 to 2000 mg/kg.

The active form is undissociated acid (pK = 4.19 at 25 °C), which is about 100 times more effective than its anion. The antimicrobial effect is probably due to inhibition of amino acid utility by microorganisms, inhibition of transport of substrates and inhibition of enzymes involved in the acetic acid metabolism, oxidative phosphorylation and the citric acid cycle.

In small amounts, benzoic acid (free or bound in esters) occurs as a natural component of foods. It is mainly present in fruits and some fermented dairy products (see Section 8.2.6.1.6).

11.2.1.1.2 Sorbic acid

(2E, 4E)-Hexa-2,4-dienoic acid, known as sorbic acid (E200), and its salts (sorbates, E201–E203) are potent inhibitors of a number of fungi, yeasts and some bacteria. Sorbic acid is used (according to the type of food) in quantities of 200–2 000 mg/kg. The active form is the undissociated acid (pK = 4.76 at 25 °C), which is roughly 10–600 times more effective than the anion. The effect is related partly to inhibition of dehydrogenases involved in the oxidation of fatty acids, sulfhydryl enzymes (it is added to the cysteine thiol group) and partly to the interference with the transport of substances through cytoplasmic membrane.

Sorbic acid has a conjugated system of double bonds, which makes it susceptible to autoxidation and nucleophilic attack, sometimes giving mutagenic products. Autoxidation of sorbic acid yields unstable hydroperoxides, which decompose and the main final products are acetaldehyde and fumaric acid monoaldehyde (β-carboxyacrolein). This aldehyde reacts with amino acids and proteins and is responsible for the browning of certain foods preserved with sorbic acid. Other products of oxidation are acrolein and crotonaldehyde. In the presence of sulfur dioxide, α-angelica lactone and 2-acetyl-5-methylfuran are produced. Nucleophiles attack the sorbic acid molecule in position C-5. With amines under conditions typical of food processing (50–80 °C) cyclic products are formed, N-alkyl-6-methyl-3,6-dihydropyrid-2-ones, resulting from a double addition reaction. The reaction with sulfur dioxide (bisulfites) and thiols yields mainly 5-substituted hex-2-enoic acids. Because sorbic acid inhibits the growth of *Clostridium botulinum* bacteria as well as the formation of nitrosamines, it has been proposed as a partial replacement for nitrite in meat curing. However, this practice may lead to other toxicological problems since sorbic acid reacts with nitrite to yield mutagenic products. At pH 3.5–4.2, the main mutagens are unstable 1,4-dinitro-2-methylpyrrole, ethylnitrolic acid and a derivative of furoxan (1,2,5-oxadiazole 2-oxide) 3-(5-methyl-4-furoxanyl)prop-2-enoic acid, which undergo decomposition to other products. Ascorbic acid completely eliminates the mutagenicity of 1,4-dinitro-2-methylpyrrole by reduction of the C-4 nitro group to a C-amino group. The 1-nitro-2-methyl-4-amino pyrrole formed is non-mutagenic (Figure 11.1).

Some microorganisms (such as *Penicillium roqueforti*) decarboxylate sorbic acid to penta-1,3-diene, which causes an off-flavour (resembling kerosene) in cheeses (Figure 11.1). The threshold concentration of penta-1,3-diene is 1 mg/kg. Also reported are cases of contaminated margarines, fruit drinks, jams and marzipan, and similar products that used pure cultures of microbial strains (such as blue cheese and fermented dairy products).

11.2.1.1.3 Parabens

Alkyl esters (methyl, ethyl, propyl and heptyl esters, previously also benzyl ester) of p-hydroxybenzoic (4-hydroxybenzoic) acid are

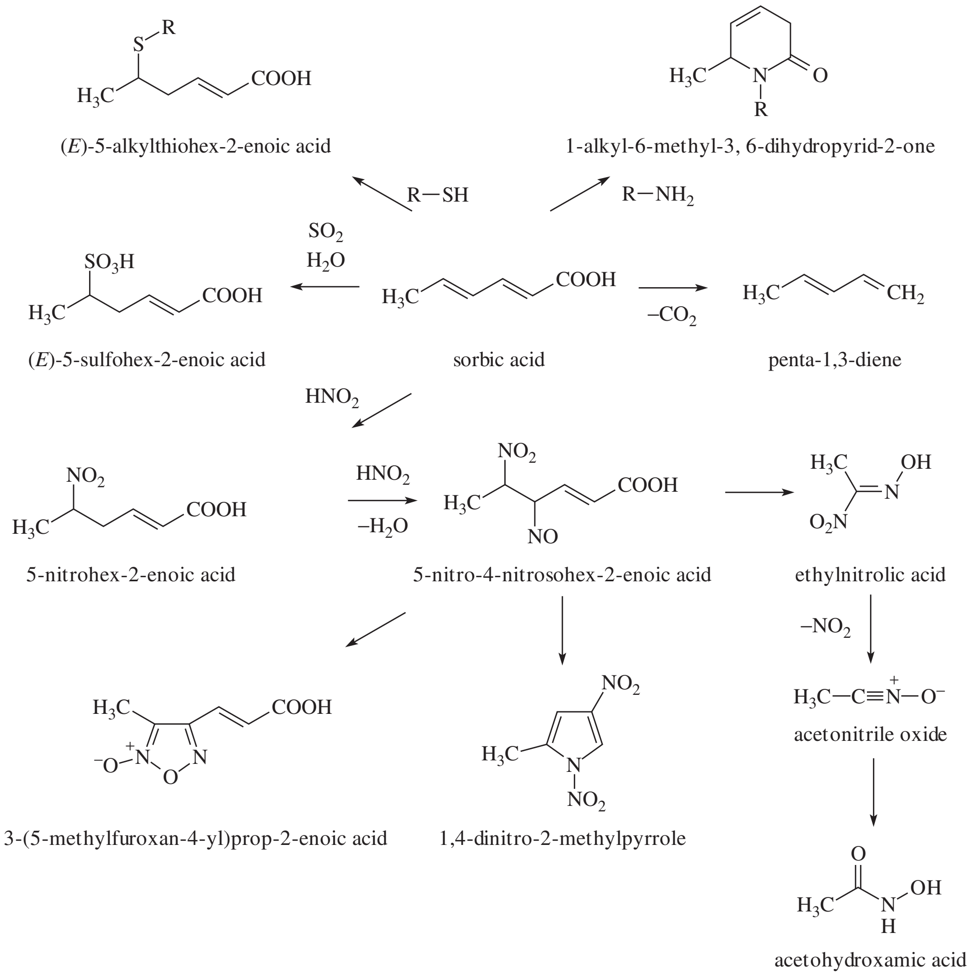


Figure 11.1 Reactions of sorbic acid in foods.

collectively called parabens. Formerly, these esters were also known as abegins or under various trade names. Unlike free acids (benzoic and sorbic acid), parabens and their salts (E209, E214–E219) are effective in less acidic and slightly alkaline environments. Their antimicrobial effectiveness increases with the length of the alkyl residue, which, on the other hand, decreases their solubility in water and thus the possibility of practical use. Salts of parabens are particularly soluble. Their effect on the taste and smell of protected foods is very small. Parabens are mainly active against fungi and yeasts and less active against gram-positive bacteria (Table 11.2). Their preservative effect is based on influencing the permeability of cell membranes. Parabens are used in amounts ranging from 500 to 1000 mg/kg.

11.2.1.1.4 Other acids and their derivatives

Formic acid ($pK = 3.75$ at 25 °C, see Section 8.2.6.1.1) is a carboxylic acid which shows the highest antimicrobial activity of all lower carboxylic acids (E236). Formic acid and formates are especially effective against bacteria and yeasts. Lactic acid bacteria and yeasts are relatively resistant. Formic acid is used for preserving acidic fruit juices or purées, whose colour is derived from anthocyanins (such as strawberries), which therefore cannot be preserved by sulfur dioxide. During thickening, formic acid is volatilised with water vapour and removed from the product. Because of its smell, its use is very limited. The other disadvantage is that formic acid partially hydrolyses pectin. In the EU, formic acid is not approved.

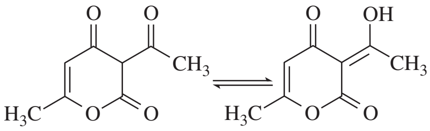
Acetic acid ($pK_a = 4.53$ at 25 °C, see Section 8.2.6.1.1) is used as a preservative and also as an acidifying agent (acidulant, E260). It is effective against yeasts and bacteria, but less so against moulds. Tolerant bacteria are acetic acid and lactic acid bacteria and gram-positive bacteria producing butyric acid under anaerobic conditions. Acetic acid is mainly used for the preparation of pickles for preserved vegetables, in production of ketchups, dressings, mayonnaises, fish and other products. The mechanism of action is the inhibition of the transport of substances through cytoplasmic membranes and inhibition of electron transport. Sodium acetate and sodium diacetate (E262, a 1 : 1 mixture of sodium acetate and acetic acid, also known as sodium hydrogen acetate) are effective preservatives against bacteria and fungi.

Dehydroacetic acid (E265, **11-1**) and its sodium salt is mainly active against fungi at higher pH values, when other acids are ineffective. In the EU, it is not approved, but in the United

Table 11.2 Antimicrobial spectrum of parabens.

Microorganism	Concentration (mg/l) causing inhibition			Microorganism	Concentration (mg/l) causing inhibition		
	Methyl	Propyl	Heptyl		Methyl	Propyl	Heptyl
Bacteria				*Fungi (yeasts and moulds)*			
Bacillus cereus	2000	125–400	12	*Candida albicans*	1000	125–250	–
Escherichia coli	2000	400–1000	–	*Saccharomyces bayanus*	930	220	–
Lysteria monocytogenes	> 512	512	–	*Saccharomyces cerevisiae*	1000	125–200	100
Pseudomonas aeruginosa	4000	8000	–	*Aspergillus flavus*	–	200	–
Salmonella typhosa	2000	1000	–	*Aspergillus* niger	1000	200–250	–
Staphylococcus aureus	4000	350–500	12	*Rhizopus nigricans*	500	125	–

States this acid is allowed for the preservation of cut or peeled squash. It was similarly used as a fungistatic agent for surface treatment of cheeses or for suppressing the microbial defects of bread and related products called ropiness or ropy bread, which is caused by the thermophilic spore-forming bacterium *Bacillus mesentericus* (a variant of *Bacillus subtilis*), whose spores survive cooking temperatures that do not exceed 100 °C inside the bread, and can germinate under favourable conditions. Bread ropiness is a result of slime casings production by these bacteria and enzymatic hydrolysis of starch and gluten. On the second or third day, the bread crumb becomes damp and sticky as a result of the formation of gummy products that stretch into strands when the bread is pulled apart. In addition to ropiness, the spoiled bread has an off-flavour, sometimes characterised as fruity or pineapple-like, and turns yellow. Formerly, when ropiness occurred, bakers acidified doughs with vinegar as a protective measure. Dehydroacetic acid has also been shown to be an effective antifungal agent against black bread moulds, particularly *Aspergillus niger* and *Rhizopus nigrificans*.



11-1, dehydroacetic acid

Propionic acid arises in Emmental cheeses by propionic acid fermentation (see Section 8.2.6.1.1). Propionic acid (E280) and its salts (E281–E283) are active as preservatives in weakly acidic medium (to pH 5 , pK = 4.87 at 25 °C), especially against moulds but less against gram-negative bacteria, while the activity against yeasts is almost completely absent. Propionic acid is mainly used as a preservative in stored animal feed and grain. It is also used for extending the shelf life of bread, tortillas and other cereal products, as it prevents the growth of moulds (as calcium propionate and sodium propionate). The inhibition of bacteria that cause bread ropiness occurs at concentrations of 2000–3000 mg/kg. The mechanism of action is mainly based on accumulation of propionic acid in the cells of microorganisms and inhibition of important enzymes.

Lactic acid (pK = 3.83 at 25 °C, see Section 8.2.6.1.3) occurs as a natural compound in many fermented products (e.g. yoghurt, fermented sauerkraut and olives). As a preservative, lactic acid (E270) is mainly used as an acidulant. The undissociated form diffuses through cell membrane of many bacteria and lowers the pH within the cell. It has a bacteriostatic effect on pathogenic bacteria *Mycobacterium bovis*, a member of the *M. tuberculosis* bacteria, the causative agent of bovine tuberculosis in cattle. Lactic acid is also effective against other bacteria, so it is used, for example, for surface decontamination of meat and in production of delicacies. Usually it is applied in combination with sodium lactate.

Fumaric acid ($pK_1 = 3.09, pK_2 = 4.60$ at 25 °C, see Section 8.2.6.1.2) is used as an acidulant and preservative (E297) for inhibition of lactic acid fermentation in wines. Fumaric acid and its esters (especially monomethyl and monoethyl esters) may slow down the formation of botulinum toxin formation in canned meat and prevent the growth of moulds in bread.

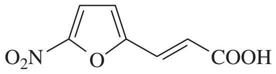
Other organic acids are important mainly as acidulants. Approved substances in the EU are citric, malic, tartaric (see Section 8.2.6.1.3) and ascorbic acids (see Section 5.14.1) and their sodium, potassium and calcium salts.

11.2.1.2 Other organic substances

In addition to carboxylic acids, their salts and esters, some other organic compounds that have antimicrobial effects can similarly be used as food preservatives. These substances include some antibiotics, enzyme lysozyme, biphenyl, *o*-phenylphenol, thiabendazole, dialkyl dicarbonates and alkylene oxides. Other substances with antimicrobial effects belong to other categories of food additives. For example, sucrose esters with palmitic and stearic acid, used as emulsifiers, are active against fungi of the genera *Aspergillus*, *Penicillium* and *Cladosporium*. Some other organic compounds, which are used for food preservation, are not considered food additives, such as sucrose, as well as different natural substances with antimicrobial effects occurring in some vegetables (such as allicin from garlic or allyl isothiocyanate from horseradish) or spices (e.g. piperine in

black pepper and capsaicin from hot peppers). Many other natural substances show antimicrobial effects, some of which are listed in Section 10.3.3.6.

During the last few decades, some previously used preservatives have been dropped due to hygienic–toxicological reasons (in some cases it was found that they were potential carcinogens) and are prohibited in the EU and other countries. Such substances include, for example, dehydroacetic acid (**11-1**) and its sodium salt (previously used to preserve jams and margarines), 8-hydroxyquinoline (for tobacco), thiourea (for fresh fruits), salicylic acid (for fruits and vegetables), 3-(5-nitrofuran-1-yl)acrylic acid (for wines, **11-2**).



11-2, 3-(5-nitrofuran-1-yl)acrylic acid

11.2.1.2.1 Antibiotics

The use of antibiotics in food is problematic, because the use of the same substances in human and veterinary medicine are not allowed. With regard to this requirement, only polypeptide antibiotics produced by lactic acid bacteria are approved in the EU and other countries (including certain strains of the genera *Lactococcus*, *Lactobacillus*, *Leuconostoc* and *Pediococcus*), known under the general common name **bacteriocins**.

Nisin

The thermoresistant polypeptide nisin (E234), produced by some strains of *Streptococcus lactis*, which is effective against gram-positive bacteria, is important as a preservative, and may occur naturally in certain cheeses as a result of fermentation processes. Gram-negative bacteria are resistant to nisin, because their cell walls are far less permeable than those of gram-positive bacteria. Its use is legally limited to certain milk products, such as aged and processed cheeses, puddings and creams. The molecule of nisin (**11-3**) is composed of 33 amino acid residues arranged in five cycles. Nisin contains some unusual amino acid residues: Abu = 2-aminobutanoic acid, Dha = dehydroalanine (2-aminoacrylic acid) and Dhb = dehydrobutyrine (2-aminocrotonic acid), which occurs as the L- or D-enantiomer, and thioether cross-linkages (*meso*-lanthionine and 3-methyllanthionine residues **2-123**) that are indicated as Ala-S-Ala and Abu-S-Ala, respectively (in which the amino-terminal moieties have the D-configuration). The peptide diplococcin produced by *Streptococcus cremoris* (*Lactococcus lactis*, ssp. *cremoris*) has a comparable antibacterial spectrum.

Natamycin

Natamycin (E235), also known as pimaricin or tennecetin (**11-4**) is a polyene macrolide substituted by sugar residues derived from 3-amino-3,6-dideoxy-β-D-mannose. It is produced by certain strains of the bacteria *Streptomyces natalensis* or *Streptococcus lactis* and is allowed for surface treatment of cheeses and durable meat products. Natamycin is active against most fungi and yeasts at concentrations of 5–10 mg/kg, but is ineffective against bacteria. Like other polyene antibiotics, natamycin inhibits fungal growth by binding to ergosterol in the plasma membrane, preventing ergosterol-dependent fusion of vacuoles, as well as membrane fusion and fission.

11.2.1.2.2 Lysozyme

The active substances against gram-positive bacteria is the enzyme lysozyme (neuramidinase) from the group of hydrolases. Lysozyme is allowed in many countries for the treatment of some dairy products and wine.

11.2.1.2.3 Biphenyl and its derivatives

Biphenyl (E230) and *o*-phenylphenol (E231), also known as 2-biphenylol (**11-5**) or its sodium salt sodium *o*-phenylphenol (E232) have been used for the surface treatment of citrus fruits. They are active against fungi.

11.2.1.2.4 Thiabendazole

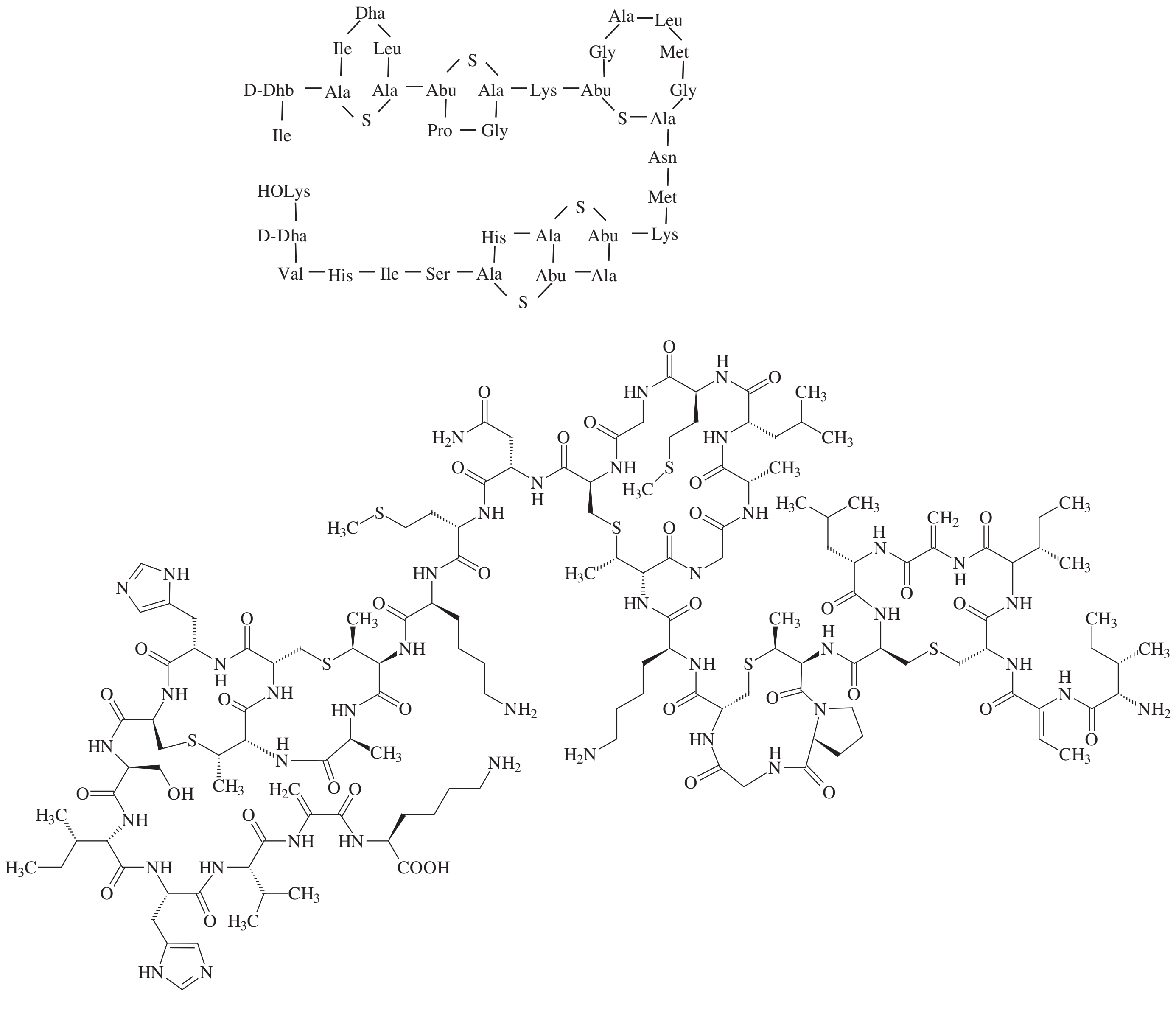
The fungicide 2-(4-thiazolyl)benzimidazole, known as thiabendazole (E233) has been used for the same purpose as biphenyl and *o*-phenylphenol and for the surface treatment of bananas.

11.2.1.2.5 Hexamethylenetetramine

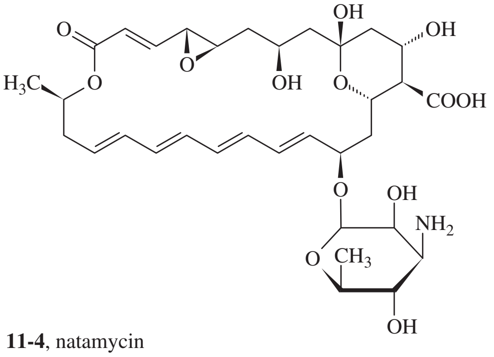
Hexamethylenetetramine (E239), also known as urotropine, hexamine, methenamine or 1,3,5,7-tetraazaadamantane (**8-57**), arises as a reaction product of formaldehyde with ammonia. Its preservation effect appears to be due to the gradual liberation of formaldehyde (E240, which is not approved in the EU) and its oxidation product formic acid under acid conditions or in the presence of proteins. The most abundant end-product of formaldehyde in cheeses preserved with hexamethylenetetramine is spinacine, 4,5,5,7-tetrahydro-3*H*-imidazo[4,5-*d*]pyridine-6-carboxylic acid (**11-6**), derived from the *N*-terminal histidine residue in γ-casein.

11.2.1.2.6 Dialkyl dicarbonates

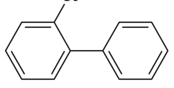
Diethyl dicarbonate (diethyl pyrocarbonate) was used in the past as a preservative for soft drinks and some alcoholic beverages (including wine). In aqueous solution, however, diethyl dicarbonate is rapidly hydrolysed to ethanol and carbon dioxide, and in alcoholic beverages it is transformed into diethyl carbonate by reaction with ethanol. In the presence of ammonium salts toxic ethyl carbamate then results, which is also known as urethane (Figure 11.2). For these reasons, diethyl dicarbonate is not approved in the EU, but the approved compound is dimethyl dicarbonate (E242), which can be used for the preservation of soft drinks, teas, herbal teas and in some countries to stabilise wine. The methyl carbamate produced from dimethtyl dicarbonate is a non-toxic substance. In treated wines it occurs in amounts up to 10 μg/l.



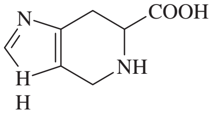
11-3, nisin



11-4, natamycin



11-5, biphenyl, R = H
o-phenylphenol, R = OH



11-6, spinacine

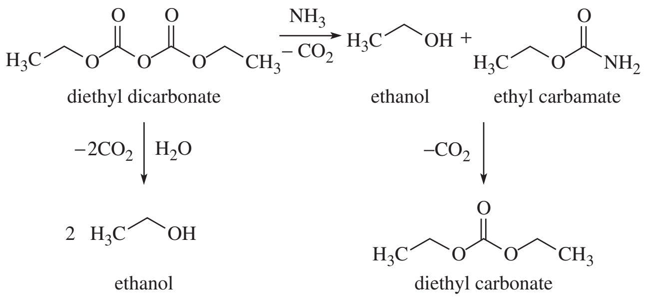


Figure 11.2 Degradation of diethyl dicarbonate in foods.

11.2.1.2.7 Alkylene oxides

Effective substances against all microorganisms (vegetative forms and spores) and also with insecticidal properties are ethylene oxide (oxirane) and propylene oxide (methyloxirane). In some countries, these compounds are approved for fumigation of foods with low water contents, where other methods of preservation are not applicable (such as sterilisation of spices, nuts, starch and flour). The modern alternative is irradiation.

Oxiranes are highly toxic alkylating agents and the hydrolysis product of ethylene oxide ethylene glycol and reaction products of oxiranes with chloride ions is likewise toxic. The latter reaction yields 2-chloroethanol, which arises from oxirane, and isomeric vicinal chloropropanols (chlorohydrins) resulting from methyloxirane (Figure 11.3). Oxiranes react with a number of other food components, such as vitamins (riboflavin, pyridoxine, niacin and folic acid) or amino acids (methionine and histidine) to form biologically inactive products.

11.2.1.3 Inorganic compounds

11.2.1.3.1 Sulfur dioxide and sulfites

Sulfur dioxide (SO_2, E220) and some of its derivatives, such as sulfites (SO_3^{2-}; E221, E225, E226), sodium bisulfite (E222) and disulfites, commonly known as pyrosulfates or metabisulfites ($S_2O_5^{2-}$; E223, E224), can be used as preservatives, but also as inhibitors of enzymatic and non-enzymatic browning reactions, bleaching agents and antioxidants. Calcium bisulfite (E227) is used as a firming agent.

Aqueous solutions of sulfur dioxide (SO_2 dissolves up to 9.5% solution at 20 °C) give rise to sulfurous acid (H_2SO_3), the anhydride of which is sulfur dioxide. Sulfurous acid dissociates into two stages ($pK_1 = 1.76, pK_2 = 7.20$). Depending on the pH of the medium, solutions of sulfurous acid contain, in addition to SO_2 and undissociated acid, bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) ions. In acidic foods of pH 3–4, bisulfites dominate:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3 \xrightleftharpoons{pK=1.81} H^+ \; HSO_3^- \xrightleftharpoons{pK=6.91} 2\; H^+ + SO_3^{2-}$$

In acidic aqueous solution, disulfite salts decompose to bisulfites and sulfur dioxide:

$$S_2O_5^{2-} + H^+ \rightarrow HSO_3^- + SO_2$$

The active form is undissociated acid, which is the only form effective against yeasts, therefore sulfur dioxide and sulfites are only effective in acidic foods (pH < 4). In some countries, sulfur dioxide solutions can be used to inhibit the growth of bacteria (although they are less sensitive against yeasts and moulds) on the surface of meat and meat products. The main applications of sulfur dioxide and sulfites concern the growth inhibition of acetic acid and lactic acid bacteria and wild yeasts in wine. The levels of $Na_2S_2O_5$or $K_2S_2O_5$ (sodium or potassium metabisulfite) and/or $KHSO_3$ (potassium bisulfite) compounds allowed by the European Union for some types of wine vary from 160 to 260 mg/l. Other applications are related to protecting the fruit from the growth of moulds. Sulfur dioxide acts against certain bacteria in concentrations of around 1–2 mg/l, which is a bacteriostatic effect, but at higher concentrations its effect is bactericidal. Concentrations of 1–10 mg/l (at pH 3.5), for example, inhibit the lactic acid fermentation of fruit products. Concentrations inhibiting yeasts (*Saccharomyces cerevisiae*) and moulds vary from 0.1 to 20 mg/l. The amounts of sulfur dioxide (sulfite) used may range, according to the type of food, from 10 to 2000 mg/kg.

Sulfur dioxide in foods yields a number of addition products with simple aldehydes, ketones and also with sugars (e.g. in wine), allyl isothiocyanate (e.g. in mustard) and a number of other food constituents in which it is reversibly bound. Aliphatic aldehydes form adducts with sulfur dioxide (bisulfites), whereas ketones are found predominantly in their free form. Reactions with sugars and their degradation products are described in Section 4.7.5.11. Sulfur dioxide also forms irreversibly bound forms, which are very stable. These products are formed with some sugar degradation products in the non-enzymatic browning reactions (see Section 4.7.1), oxidised phenols (such as chlorogenic acids, see Section 9.12.4) and also with proteins and peptides containing disulfide bonds, which are cleaved with the formation of thiosulfates (*S*-sulfonates, R–S–SO_3^-). In this reaction the cleavage of disulfide

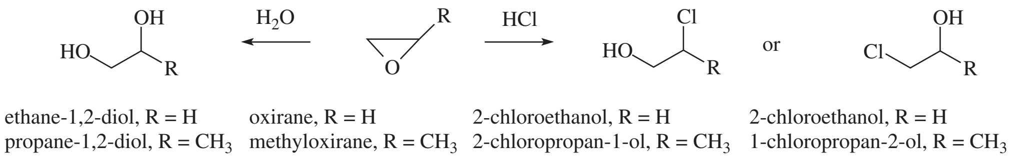


Figure 11.3 Reactions of oxiranes with water and hydrogen chloride.

bonds is produced by a nucleophilic displacement mechanism to form thiols and *S*-sulfonates:

$$R–S–S–R + SO_3^{2-} \rightarrow R–S–SO_3^- + RS^-$$

Sulfur dioxide (bisulfites) also reacts with thiamine yielding inactive compounds (see Section 5.6.6), forms adducts with riboflavin, nicotinamide, vitamin K, inhibits ascorbic acid oxidation (see Section 5.14.6.1.6) and reacts with ascorbic acid degradation products, reduces *o*-quinones produced in enzymatic browning reactions back to 1,2-diphenols (see Section 9.12.4), causes decolourisation of fruit anthocyanins (see Section 9.4.1.5.7) and reacts with synthetic azo dyes to form coloured or colourless products (see Section 11.4.1.3.2). Sulfur dioxide also reacts with pyrimidine bases *in vitro*, specifically with cytosine and 5-methylcytosine. Important reactions of sulfur dioxide are shown in Figure 11.4.

Sulfur dioxide or bisulfites are rarely used in combination with nitrites, because their reactions in acidic media yield sulfonates of either hydroxylamine or ammonia, which destroys the preservative activity of the individual additives. The reaction of alkali metal nitrites with bisulfite at lower temperatures leads to the formation of hydroxylamine *N*, *N*-disulfonate, $HON(SO_3)_2^{2-}$, which is hydrolysed to hydroxylamine *N*-sulfonate, $HONHSO_3^-$ and further to hydroxylamine (H_2NOH). At elevated temperatures complete substitution proceeds with the formation of ammonia *N*, *N*, *N*-trisulfonate, $N(SO_3)_3^{3-}$, which is hydrolysed to sulfamate, $H_2NSO_3^-$. Subsequent reaction of sulfamate with nitrous acid leads to production of bisulfate ion and nitrogen gas:

$$NO_2^- + 2HSO_3^- \rightarrow HON(SO_3)_2^{2-} + HO^-$$
$$HON(SO_3)_2^{2-} + H_2O \rightarrow HONHSO_3^- + HSO_4^-$$
$$HONHSO_3^- + H_2O \rightarrow H_2NOH + HSO_4^-$$
$$HON(SO_3)_2^{2-} + HSO_3^- \rightarrow N(SO_3)_3^{3-} + H_2O$$
$$N(SO_3)_3^{3-} + 2H_3O^+ \rightarrow H_2NSO_3^- + 2H_2SO_4$$
$$H_2NSO_3^- + HNO_2 \rightarrow N_2 + HSO_4^- + H_2O$$

11.2.1.3.2 Nitrites

In addition to use for meat colour stabilisation (see Section 9.3.1.5.2), nitrites (NO_2^-, E249 and E250), together with salt (sodium chloride), can also be used as antimicrobial agents. Their use is especially important in non-sterile meat products, as they inhibit the growth of *Clostridium botulinum* bacteria. The efficiency of the inhibition depends on pH, because it is proportional to the concentration of nitrous acid (HNO_2) formed from nitrites. Nitrates (NO_3^-) may also be used as preservatives, namely sodium nitrate (E251), known as Chile saltpetre, and potassium nitrate (E252), or saltpetre, that are reduced to nitrites by microorganisms.

11.2.1.3.3 Boric acid and its salts

Boric acid (H_3BO_3, E284) and disodium tetraborate ($Na_2B_4O_7$), known as borax (E285) are permitted for use as preservatives in some countries and in the EU are only approved to preserve caviar. Their preservative effect is based on inhibition of decarboxylases of amino acids and inhibition of phosphate metabolism.

11.2.1.3.4 Sodium chloride

Sodium chloride (NaCl) is commonly used in combination with other preservatives and preservation methods, but is not classified as a food additive. The antimicrobial activity of sodium chloride is related to its ability to reduce water activity, thus creating unfavourable conditions for microbial growth (sucrose is similarly active). The sensitivity of microorganisms varies considerably. Intolerant bacteria can be inhibited by a level of 10 g/kg, mesophilic bacteria and psychrotropic gram-negative rods tolerate

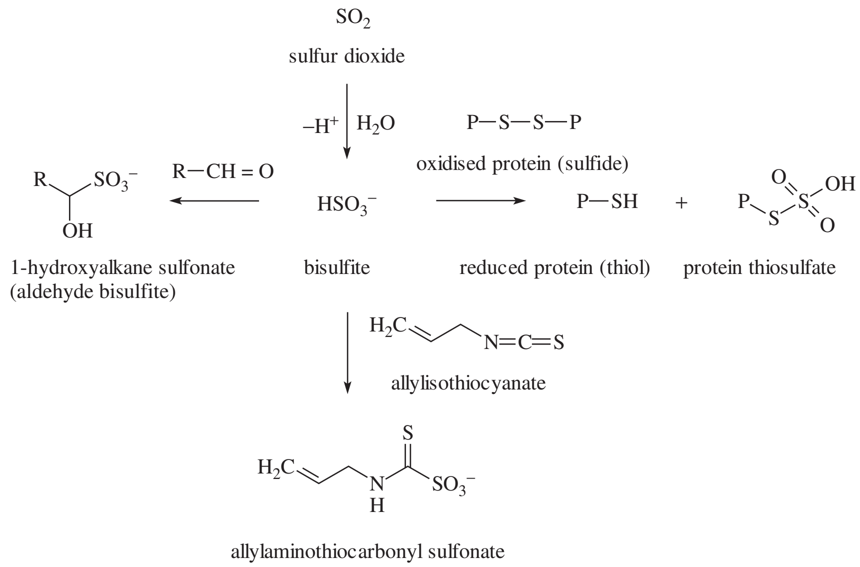


Figure 11.4 Important reactions of sulfur dioxide in foods.

concentrations 6–10 times higher, lactic bacteria survive in an environment where the concentration of sodium chloride ranges from 60 to 150 g/kg and spore-forming bacteria can even tolerate sodium chloride concentrations of 160 g/kg.

11.2.1.3.5 Other inorganic substances

Inorganic compounds exhibiting antimicrobial activity also include hydrogen peroxide, phosphates or carbon dioxide, which is effective against fungi and gram-negative psychrotropic bacteria, but lactic acid bacteria and anaerobes are less inhibited.

11.2.1.4 Legislation

Compounds that are conditionally permitted for food preservation in the EU are listed in Table 11.3. All preservatives may only be used for the preservation of listed foods and to the maximum amount allowed.

Benzoic acid and its salts can be used for jams, jellies and marmalades, either alone or in mixtures with sorbic acid. Benzoic acid in quantities of up to 30 mg/kg in fermented dairy products is not considered a food additive, as it arises from the hippuric acid (see **2-17**). For example, the maximum permissible level of benzoic acid for cooked red beet is 2000 mg/kg, and for cooked crustaceans and molluscs 1000 mg/kg. Sorbic acid alone is allowed, for example, for potato dough, pre-fried potato slices and processed cheeses (2000 mg/kg), dried fruit and gnocchi (1000 mg/kg) and wines (200 mg/l). Spirits with less than 15% alcohol by volume can contain a mixture of benzoic acid (200 mg/l) and sorbic acid (200 mg/l), olives and olive-based preparations may contain benzoic acid (500 mg/kg) and sorbic acid (1000 mg/kg). Sorbic acid with *p*-hydroxybenzoates or their salts may be used, for instance, in preserved jelly coatings of meat products and cereal- or potato-based snacks and coated nuts (1000 mg/kg).

The use of propionic acid and its salts are allowed for pre-packed sliced bread and rye bread (3000 mg/kg), and for energy reduced bread, partially baked, pre-packed bread and pre-packed fine bakery wares (including flour confectionery) with a water activity of more than 0.65 (2000 mg/kg). Fermented milk products (Emmental type cheeses) contain natural propionic acid arising in the fermentation process, which is not regarded as a food additive.

Nisin can be used for semolina and tapioca puddings and similar products (3 mg/kg), ripened and processed cheeses 12.5 mg/kg) and Mascarpone cheese (10 mg/kg). Natamycin may be used for surface treatment of hard, semi-hard and semi-soft cheeses and dried, cured sausages (1 mg/dm^2 surface up to a depth of 5 mm). Lysozyme is used in *quantum satis* (a Latin term meaning the amount, which is needed), for example, to preserve ripened cheeses.

Table 11.3 List of current EU approved preservatives and their E-numbers.

E-number	Name[a]	E-number	Name[a]
E200	Sorbic acid	E228	Potassium hydrogen sulfite
E202	Potassium sorbate	E230	Biphenyl (diphenyl)
E203	Calcium sorbate	E234	Nisin
E210	Benzoic acid	E235	Natamycin
E211	Sodium benzoate	E239	Hexamethylenetetramine
E212	Potassium benzoate	E242	Dimethyl dicarbonate
E213	Calcium benzoate	E249	Potassium nitrite
E214	Ethyl *p*-hydroxybenzoate	E250	Sodium nitrite
E215	Sodium ethyl *p*-hydroxybenzoate	E251	Sodium nitrate
E218	Methyl *p*-hydroxybenzoate	E252	Potassium nitrate
E219	Sodium methyl *p*-hydroxybenzoate	E280	Propionic acid
E220	Sulfur dioxide	E281	Sodium propionate
E221	Sodium sulfite	E282	Calcium propionate
E222	Sodium hydrogen sulfite	E283	Potassium propionate
E223	Sodium metabisulfite	E284	Boric acid
E224	Potassium metabisulfite	E285	Sodium tetraborate (borax)
E226	Calcium sulfite	E1105	Lysozyme
E227	Calcium hydrogen sulfite		

[a]Directive No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners.

Biphenyl is only used for the surface treatment of citrus fruits. Hexamethylenetetramine is only approved for preservation of Provolone cheese that originates in Casilli near Napoli. A variant of Provolone cheese is also produced in North America and Japan. In the past, hexamethylenetetramine was also used as a preservative for collagen casings and fish products. Dimethyl dicarbonate is allowed for non-alcoholic flavoured drinks, alcohol-free wine and liquid-tea concentrates at the amount of 250 mg/l.

The maximum level of sulfur dioxide and sulfites (expressed as SO_2) differ significantly in the individual foodstuffs. For example, SO_2 content in peeled potato can be 50 mg/kg, 100 mg/kg in processed potatoes (including frozen and deep-frozen potatoes), potato dough, dried mushrooms and jams, jellies and marmalades made with sulfited fruit, 200 mg/kg in dried tomato, 400 mg/kg in dried white vegetables and dehydrated potato, 450 mg/kg in burger meat with a minimum vegetable and/or cereal content of 4% and in breakfast sausages, 600 mg/kg in dried apples and pears, 1000 mg/kg in dried bananas and 2000 mg/kg in dried apricots, peaches, grapes, prunes and figs. Under EU law, any wine containing more than 10 mg/l of SO_2 must be labelled as 'containing sulfites'. The maximum permitted levels of SO_2 are 160 mg/l in red wines, 200 mg/l in rose and white wines and 400 mg/l in sweet wines. In beer, including low-alcohol and alcohol-free beer, where SO_2 arises through yeast activity, its content may be up to 20 mg/l, and even 50 mg/l in beer when there is a second fermentation in the cask.

The maximum amount of nitrites that may be added during the manufacture of meat products and sterilised meat products is 150 mg/kg and 100 mg/kg (expressed as $NaNO_2$), respectively. The maximum residual levels are also set. Potassium and sodium nitrates can be used for non-heat-treated meats at a level of 150 mg/kg, and at 300 mg/kg for traditional immersion cured meat speciality products (such as, British Wiltshire bacon and Wiltshire ham, German *Rohschinken* and Spanish *jamón curado*).

Boric acid and borax are only allowed for caviar at a level of 4 g/kg (expressed as boric acid).

11.2.1.5 Health assessment

Benzoic acid and benzoates have low toxicity, and an amount of 5–10 g administered over several days does not have adverse effects (ADI = 5 mg/kg bodyweight). This is because there is an effective detoxification mechanism, consisting of conjugation of benzoates with glycine to hippuric acid, which is excreted in the urine. Around 66–95% of benzoates can be removed in this way, with the rest taking the form of glucuronate. Some individuals, however, show increased sensitivity to benzoic acid. Sorbic acid (also sorbates) is regarded as one of the least toxic preservatives (ADI = 25 mg/kg bodyweight), but in cosmetic and pharmaceutical products may irritate the skin in susceptible individuals. Parabens and the corresponding free acid are even less toxic than sorbic acid (ADI = 70 mg/kg bodyweight), but they are rapidly hydrolysed and the free acid is excreted as a conjugate with glycine and glucuronic acid. Parabens exhibit local anaesthetic effects and may cause dermatitis in susceptible individuals. The intake of propionic acid is not limited. The use of formic acid (ADI = 3 mg/kg bodyweight) is prohibited in food preservation in some countries (including the EU) and formic acid (as well as methanol from which formic acid arises through the action of alcohol dehydrogenase via formaldehyde) is known to be toxic to the optic nerve (see Section 8.2.2.2).

Nisin is authorised for food preservation in the EU. For nisin, an ADI equal to 0.13 mg/kg of body weight, and for natamycin an ADI equal to 0.3 mg/kg of body weight have been set. In 2003, the European Parliament announced that nisin should not be used because of possible worsening effect of antibiotics on humans, but the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC Panel) approved the use of nisin as a food additive in 2006. This substance is also permitted in the United States.

For the antifungal phenols, ADI = 0.05 mg/kg body weight for biphenyl and 0.2 mg/kg body weight for *o*-phenylphenol and its sodium salt have been determined. Hexamethylenetetramine may liberate free formaldehyde in the stomach. Both hexamethylenetetramine (ADI = 0–0.15 mg/kg body weight) and formaldehyde (ADI = 0.15 mg/kg body weight) have been shown to act as mutagens in *Drosophila*. Acute and short-term toxicological studies on spinacine led to an ADI = 3 mg/kg body weight. It was concluded that there is no appreciable health risk from consumption of cheese made using formaldehyde (Grana Padano cheese) or hexamethylenetetramine (Provolone cheese). The ADI value for dimethyl dicarbonate was not determined, as this ester decomposes when dissolved in the product.

Bisulfites are oxidised in the body by sulfite oxidase (sulfite: ferricytochrom c oxidoreductase) to sulfates, which are excreted in the urine. The enzyme activity is individual, and toxic effects of sulfur dioxide and sulfites are therefore variable. Some individuals tolerate amounts of up to 50 mg/kg, while in sensitive individuals such concentrations will cause headaches, nausea and diarrhoea. In asthmatics receiving steroids, sulfites may cause allergic reactions (ADI = 0.7 mg/kg body weight).

Health and toxicological evaluation of nitrates and nitrites is given in detail in Section 6.7.1.2. ADI values of 3.7 mg/kg body weight for nitrates and 0.07 mg/kg body weight for nitrites have been set. Nitrites can induce tumours either directly through effects on immune functions or indirectly via carcinogenic *N*-nitrosamines produced from nitrites and secondary amines. Repeated consumption of higher amounts of nitrites may cause methaemoglobinaemia, especially in children.

Boric acid and borax (ADI = 0.1 mg/kg body weight) resorb rapidly in the body and are slowly excreted. In comparison with other acids, which act as preservatives, these compounds are more toxic, but no side effects are known from foods. Concentrations of boric acid (borates) in pharmaceutical preparations are much higher and may cause several side effects. Chronic administration causes bleeding, dermatitis and anaemia.

11.2.2 Antioxidants

Antioxidants are substances that prolong the shelf life of foods by protecting them against deterioration caused by oxidation, which is reflected in rancidity in fats and other easily oxidising food components. Lipid oxidation causes other chemical changes in foods that negatively affect their nutritional value (oxidation of vitamins), sensory value (oxidation of flavour-active components

Table 11.4 List of current EU approved antioxidants and their E-numbers.

E-number	Name[a]	E-number	Name[a]
E300	Ascorbic acid[b]	E311	Octyl gallate
E301	Sodium ascorbate	E312	Dodecyl gallate
E302	Calcium ascorbate	E315	Erythorbic (isoascorbic) acid
E304	Ascorbic acid fatty acid esters	E316	Sodium erythorbate (isoascorbate)
E306	Tocopherols (natural mixture)	E319	*tert*-Butylhydroquinone (TBHQ)
E307	α-Tocoferol	E320	*tert*-Butylhydroxyanisol (BHA)
E308	γ-Tocoferol	E321	*tert*-Butylhydroxytoluene (BHT)
E309	δ-Tocoferol	E392	Rosemary extracts
E310	Propyl gallate	E586	4-Hexylresorcinol

[a]Directive No. 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners.
[b]Ascorbic acid (E300) is classified as a food supplement.

and pigments) and hygienic–toxicological quality (some oxidation products may be toxic). On the other hand, the oxidation of essential fatty acids also generates desirable aromas in certain foods (fruits, vegetables and mushrooms (see Section 3.8.1.8.5).

11.2.2.1 Classification

Antioxidants interfere with the process of oxidation of lipids and other oxylabile compounds so that they:

- react with free radicals (**primary antioxidants**) or reduce the resulting hydroperoxides (**secondary antioxidants**)
- bind to catalytically active metal complexes
- eliminate the oxygen present.

The reaction mechanism is detailed in the section dealing with the oxidation of lipids (see Section 3.8.1.8). The primary antioxidants include all authorised substances: ascorbic acid and erythorbic (isoascorbic) acid and their derivatives, tocopherols and phenolic antioxidants (Table 11.4). The secondary antioxidants include, for example, cysteine, cysteine-containing peptides (such as glutathione), methionine, lipoic acid and other naturally occurring compounds, which are not however used as antioxidants. A synthetic secondary antioxidant used in the past was 3,3′-thiodipropionic acid dilaurate (**11-7**).

Equally important is the classification of antioxidants according to their origin, through which are identified:

- **natural antioxidants**
- **synthetic antioxidants**.

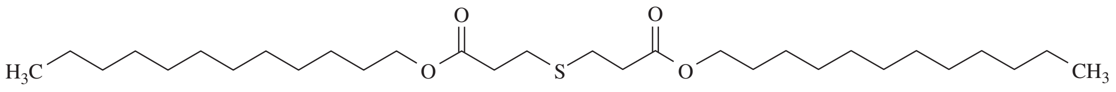
The authorised natural antioxidants include tocopherols, which today are usually synthesised. A variety of other natural antioxidants are present in a number of essential oils and other fats (especially in spices). However, they do not usually have a constant composition, tend to be less effective and are more expensive than synthetic antioxidants.

According to their structure the following may be recognised:

- **phenolic** antioxidants (of approved natural substances including tocopherols and phenolic antioxidants, but also a number of other compounds present in foods, spices and other natural materials);
- **enediols** (of approved compounds, enediols include ascorbic and erythorbic acids, their salts and other derivatives);
- **other substances**.

11.2.2.2 Mechanism of action

The mechanism of action of ascorbic acid and its derivatives is given in Section 5.14.6.1.4, and the mechanism of action of tocopherols is described in Section 5.4.6. Phenolic compounds (Ar–OH) as primary antioxidants may interfere with the oxidation of lipids (R–H) in a reaction in competition with the propagation phase reaction of autoxidation. They react with hydroperoxyl radicals



11-7, didodecyl-3,3′-thiodipropionate

($ROO^\bullet$) produced by lipid oxidation, or with alkoxyl radicals ($RO^\bullet$) arising by decomposition of lipid hydroperoxides. They provide these radicals with hydrogen, thereby interrupting the radical chain autoxidation reaction. The resulting products are phenoxyl (aryloxyl) radicals of antioxidants:

$$\text{Ar–OH} + \text{R–O–O}^\bullet \rightarrow \text{R–O–OH} + \text{Ar–O}^\bullet \text{ or}$$
$$\text{Ar–OH} + \text{R–O}^\bullet \rightarrow \text{R–OH} + \text{Ar–O}^\bullet$$

These free radicals react with hydroperoxyl and alkoxyl radicals of oxidised fatty acids in the termination phase of the reaction:

$$\text{R–O–O}^\bullet + \text{Ar–O}^\bullet \rightarrow \text{R–O–Ar} + \text{O}_2$$
$$\text{R–O}^\bullet + \text{Ar–O}^\bullet \rightarrow \text{R–O–O–Ar}$$

The reaction mechanism is shown in Figure 11.5. Phenoxyl radicals, however, cannot enter the chain radical reaction and cannot initiate the fission of other lipid molecules. These reactions, however, may occur at high concentrations of an antioxidant, which then acts as a pro-oxidant as follows:

$$\text{Ar–O}^\bullet + \text{R–O–OH} \rightarrow \text{Ar–OH} + \text{R–O–O}^\bullet$$
$$\text{Ar–O}^\bullet + \text{R–H} \rightarrow \text{Ar–OH} + \text{R}^\bullet$$

The relative stability and low reactivity of phenoxyl radicals is associated with the unpaired electron delocalisation in the aromatic system. The attack of atmospheric oxygen can be therefore very difficult. This situation is apparent, for example, in unsubstituted phenol, which is ineffective as an antioxidant. Recombination of phenoxyl radicals produces a number of dimeric and oligomeric products (Figure 11.6). Reported products from oxidation of phenol with molecular oxygen and ozone include a range of aromatic compounds, such as *o*-benzoquinone, *p*-benzochinone, catechol and hydroquinone and aliphatic compounds, such as maleic anhydride, maleic, fumaric, propionic, oxalic, glyoxylic, acetic and formic acid, carbon dioxide and carbon monoxide.

Substitution of phenol by alkyl groups in the *ortho* or *para* positions increases (by the conjugation effect) the density of electrons on the OH group, which increases the ability of phenol to react with free radicals. Stability of the resulting phenoxyl radical increases further in the presence of bulky substituents in the *ortho* position. Such antioxidants include the phenolic synthetic antioxidants BHA and BHT.

The antioxidant activity of phenol is also increased by the presence of additional hydroxyl group in the *ortho* or *para* positions. An example of such an antioxidant is TBHQ. The effectiveness of 1,2-dihydroxybenzene derivatives is attributed to a phenoxyl radical stabilised by an intramolecular hydrogen bond (**11-8**). The activity of 2-methoxyphenol is lower, because the generated radical cannot be stabilised by a hydrogen bond. The antioxidant activity of 1,2- and 1,4-dihydroxybenzene is partly caused by the fact that the semi-quinone radical can be further oxidised to the corresponding *o*-quinone or *p*-quinone, respectively, by reaction with another lipid radical (Figure 11.7) or may disproportionate to the corresponding quinone and hydroquinone.

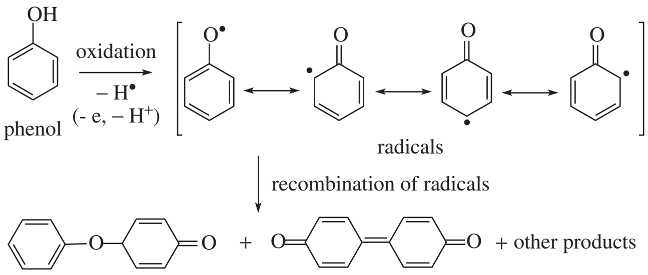


Figure 11.6 Mechanism of phenol oxidation.

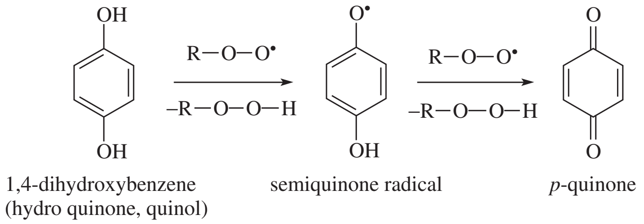


Figure 11.7 Oxidation of 1,4-dihydroxybenzene.

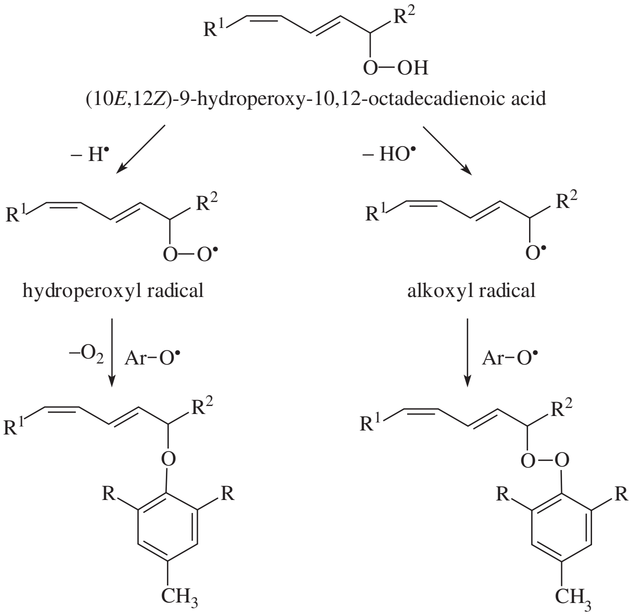
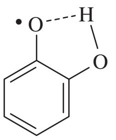


Figure 11.5 Reaction of free antioxidant radical (BHT) with free radical derived from oxidised linolenic acid
R = *tert*-butyl, $R^1 = [CH_2]_4\text{-}CH_3$, $R^2 = [CH_2]_7\text{-}COOH$.



11-8, stabilised radical of 1,2-dihydroxybenzene

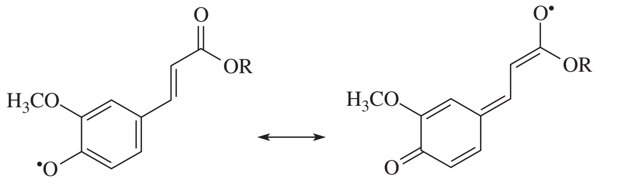


Figure 11.8 Stabilised radicals of 4-hydroxycinnamic acids.

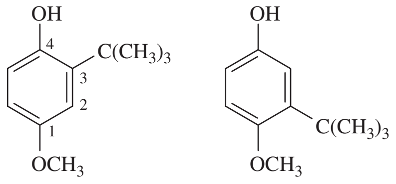
Primary antioxidants also include phenolic acids, in particular the substituted cinnamic acids and their esters (depsides), glycosides and amides. The phenoxyl radical of very effective 4-hydroxysubstituted cinnamic acids is stabilised by resonance (Figure 11.8). Many flavonoid substances are also primary antioxidants. Particularly effective compounds are chalcones, which provide resonance stabilised radicals (Figure 11.9).

11.2.2.3 Synthetic antioxidants

Modern antioxidant technology is merely about 70 years old. Only four synthetic antioxidants are widely used in foods, namely the phenolic antioxidants BHA, BHT and diphenol TBHQ (which are antioxidants of low polarity), while more polar antioxidants are esters of gallic acid (gallates) and esters of ascorbic acid. Polar antioxidants are ascorbic and erythorbic acids and their salts. Ascorbic acid, its derivatives and its analogue erythorbic (isoascorbic) acid are described in Section 5.13.1.

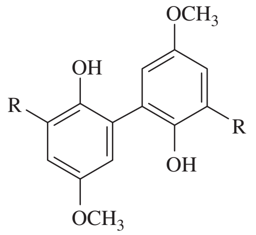
11.2.2.3.1 BHA

Commercial butylated hydroxyanisole (BHA, E320) is a mixture of two isomers. The mixture contains approximately 90% (at least 88%) 3-*tert*-butyl-4-hydroxyanisole (3-BHA) and about 10% 2-*tert*-butyl-4-hydroxyanisole (2-BHA, **11-9**). BHA is particularly effective for the protection of lipids containing fatty acids with shorter chains (in coconut and palm kernel oils) and in the aroma and colour of essential oils. Like BHT, BHA is often used in packaging materials, where it can migrate into food. Both compounds may (in contrast to the low volatile gallates) show slight odour reminiscent of phenols. BHA exhibits synergism with BHT and gallates, and in comparison with BHT it displays a somewhat higher so-called carry-through effect, which means that it is also effective as an antioxidant in the final heat treated product.

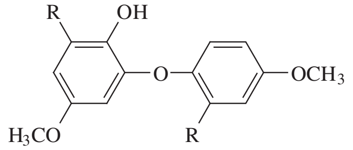


11-9, 3- *tert*-butyl-4-hydroxyanisol and 2-*tert*-butyl-4-hydroxyanisol (BHA)

As with all other antioxidants, BHA undergoes some transformation reactions during oxidation of lipids. The most common products are dimers, biphenyls (**11-10**) and their ethers (**11-11**). Most primary oxidation products still preserve the antioxidant activity.



11-10, 2,2′-dihydroxy-5,5′-dimethoxy-3, 3′-di-*tert*-butylbiphenyl, R = *tert*-butyl



11-11, 2′–,3-di-*tert*-butyl-2-hydroxy-4′, 5-dimethoxydiphenylether, R = *tert*-butyl

11.2.2.3.2 BHT

Butylated hydroxytoluene (BHT, also formerly known as Ionol, E321) is 3,5-di-*tert*-butyl-4-hydroxytoluene (**11-12**). In comparison with BHA, BHT is somewhat more effective as an antioxidant of animal fats. The formation of a BHT radical and its reactions are shown in Figure 11.10.

Important degradation products of BHT, which are also active as antioxidants, are 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (**11-13**) and 3,5,3′5′-tetra-*tert*-butyl 4,4′-dihydroxy-1,2-diphenylethane

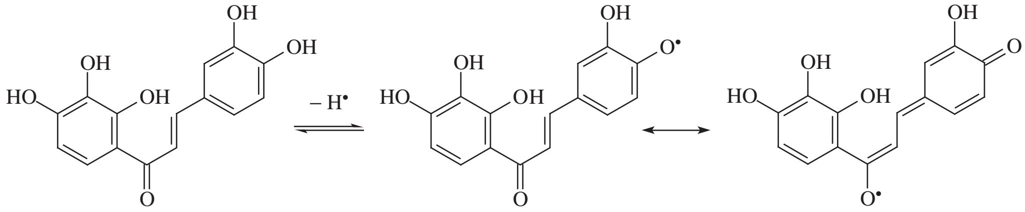


Figure 11.9 Stabilised radicals of chalcones.

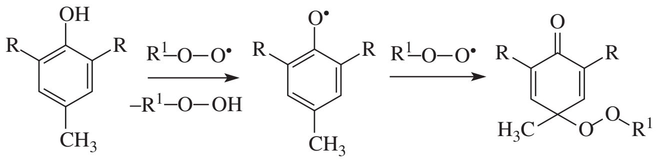
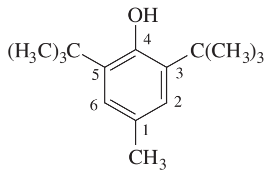
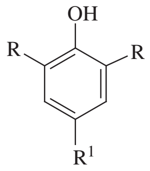


Figure 11.10 Reactions of BHT radical with hydroperoxyl radicals (R = *tert*-butyl).

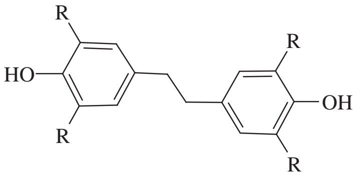
(**11-14**). In addition to these products, lower amounts of other phenols (**11-13**), quinones (**11-15**) and stilbenes (**11-16**) are formed. In addition to these products, mixed products (**11-17**) arise in mixtures of BHT and BHA.



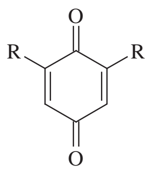
11-12, BHT



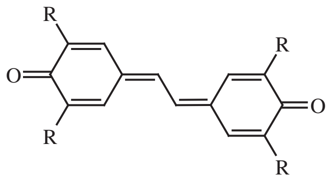
11-13, 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, R = *tert*-butyl, R^1 = CH = O
3,5-di-*tert*-butyl-4-hydroxybenzylalcohol, R = *tert*-butyl, R^1 = CH_2OH



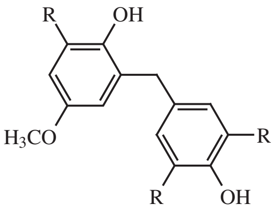
11-14, 1,2-di (3,5-di-*tert*-butyl-4-hydroxyphenyl)ethane, R = *tert*-butyl



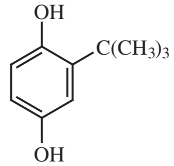
11-15, 2,6-di-*tert*-butylbenzoquinone, R = *tert*-butyl



11-16, 3,5,3´,5´-tetra-*tert*-butylstilbenequinone, R = *tert*-butyl



11-17, 3,3′ ,5′ -tri-*tert*-butyl-5-methoxy-2,
4′ -dihydroxydiphenylmethane, R = *tert*-butyl

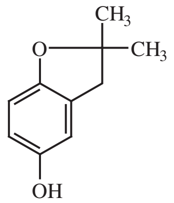


11-18, TBHQ

11.2.2.3.3 TBHQ

TBHQ (E319), 2-*tert*-butylhydroquinone (**11-18**), is the only diphenol used as an antioxidant. TBHQ is more effective in vegetable oils than BHA and BHT and is one of the best antioxidants designated for fats used for frying. Its carry-through effect is comparable with that of BHA. A further increase of TBHQ antioxidant activity, especially for the protection of vegetable oils, is possible in combination with chelating agents (such as citric acid). For example, a ternary mixture of TBHQ, monoacylglycerol citrate and ascorbyl palmitate exhibits high thermal stability and provides optimum protection in oils during high-temperature processing. In European countries and Japan, the addition of TBHQ to foods is not allowed.

As the diphenol antioxidant, TBHQ reacts with hydroperoxyl radicals to form semi-quinone radicals stabilised by resonance. These intermediates yield dimers and the original hydroquinone regenerates by dismutation. The intermediates can also react with other lipid radicals. The reaction is shown in Figure 11.11. All degradation products of TBHQ exhibit antioxidant activity, 2,3-dihydro-2,2-dimethylbenzofuran-5-ol (**11-19**) and 2-(2-hydroxy-2-methyl-1-propyl)hydroquinone (**11-20**) have even higher antioxidant activity than TBHQ.



11-19, 2,3-dihydro-2,2-dimethylbenzofuran-5-ol

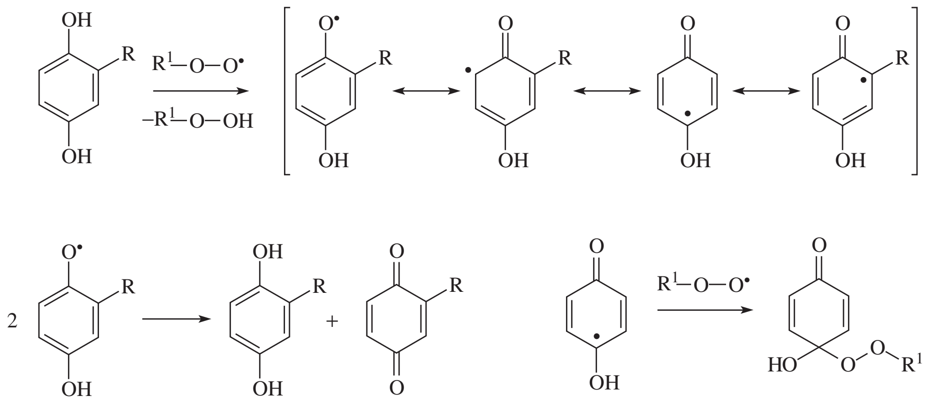
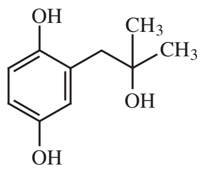


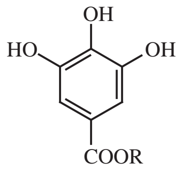
Figure 11.11 Reactions of TBHQ radicals with hydroperoxyl radicals (R = *tert*-butyl).



11-20, 2-(2-hydroxy-2-methyl-1-propyl)hydroquinone

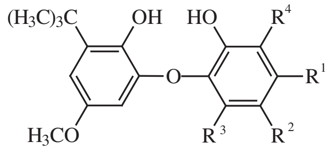
11.2.2.3.4 Gallates

Various esters of gallic acid, gallates (**11-21**), are found in small amounts in foods of plant origin. Only three esters can be used as antioxidants in the EU: propyl gallate (E310), octyl gallate (E311) and dodecyl gallate (E312). Propyl gallate is the only gallic acid ester permitted in foods in the United States and Canada. The antioxidant activity of gallates, which are more polar compounds than BHA, BHT and TBHQ, is higher in anhydrous fats or fats with minimum moisture, which is related to the solubility of gallates in the two phases, the oil and water. Propyl gallate is therefore suitable, for example, for the stabilisation of animal fats (e.g. lard and tallow). Gallates are more soluble in emulsions, but are less active than phenolic antioxidants BHA and BHT.



11-21, propyl gallate, R = $CH_2CH_2CH_3$
octyl gallate, R = $CH_2[CH_2]_6CH_3$
dodecyl gallate, R = $CH_2[CH_2]_{10}CH_3$

Propyl gallate is a relatively unstable compound, and is therefore not suitable for fats used for frying (where the temperature exceeds 190 °C). For the same reason, propyl gallate has a weak carry-through effect (unlike octyl gallate and dodecyl gallate). Propyl gallate forms blue–black complexes with iron and copper ions; therefore it is always used in combination with chelating agents (such as citric acid). Gallates exhibit synergism with BHA and BHT, but to use them together with TBHQ is illegal in the United States. One of the major degradation products arising from propyl gallate is ellagic acid (see Section 8.3.5.1), which also has antioxidant properties. In mixtures of propyl gallate with BHA, in addition to degradation products of propyl gallate and BHA, mixed dimers also arise with activity comparable to that of propyl gallate (**11-22**).

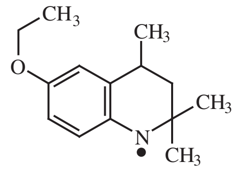


11-22, propyl-3,5-dihydroxy-4-(2-hydroxy-5-methoxy-3-*tert*-butylphenoxy)benzoate, R^1 = $COOCH_2CH_2CH_3$, R^2 = R^4 = H, R^3 = OH
propyl-3,4-dihydroxy-5-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy)benzoate, R^1 = R^3 = H, R^2 = $COOCH_2CH_2CH_3$, R^4 = OH

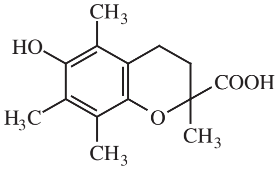
11.2.2.3.5 Other antioxidants

Antioxidants with a nitrogen heterocycle (such as dihydropyridine or dihydroquinoline derived compounds) are far less frequently used, because of their higher toxicity. An example of such an antioxidant is santokin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline), which is used by the feed industry. The active form in fats is a free radical (**11-23**). The water-soluble analogue of α-tocopherol, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid with the trade name trolox (**11-24**) has found use in biological, biochemical and food research. It is mainly used as a standard for measuring antioxidant capacities of other compounds. Many other antioxidants are used in cosmetics, the petrochemical industry and elsewhere.

Synthetic antioxidants (4-hexylresorcinol, see Section 10.2.3.4) and natural antioxidants approved in the EU as food additives, such as ascorbic acid, its salts, fatty acid esters and analogues (erythorbic acid) and tocopherols are included in Chapter 5. Natural compounds with antioxidative activity occurring in herbs and spices, including the extracts of rosemary (E392), are addressed in Section 10.3.3.7.



11-23, santokin



11-24, trolox

11.2.2.4 Legislation

Substances with antioxidant effects (Table 11.4) that may be used in food production in the necessary quantities include fatty acids esters of ascorbic acid (palmitate, stearate, see Section 5.14.4), natural products containing tocopherols and synthetic α-, β-, γ- and δ-tocopherol, which are identical to natural tocopherols, and rosemary extracts.

Erythorbic acid and sodium erythorbate may be used for preserved and semi-preserved fish products and frozen and deep-frozen fish at a concentration of 1500 mg/kg. The amount of these antioxidants allowed for preservation of cured and preserved meat products is only 500 mg/kg (expressed as erythorbic acid). The use of 4-hexylresorcinol (4-hexyl-1,3-benzenediol) is permitted up to 2 mg/kg as a colour retention agent for treatment of crustacean meat (fresh and frozen), namely to prevent black spots arising by enzymatic browning reactions, known as shrimp melanosis,

To a limited extent synthetic esters of gallic acid (propyl gallate, octyl gallate and dodecyl gallate) and phenolic antioxidants – butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) - may be used. When combinations of these antioxidants are used, the individual levels must be reduced proportionally, so that the sum of the individual antioxidants does not exceed the maximum amount of the individual antioxidants allowed for that food. For example, the maximum permissible amount of gallates and BHA is 200 mg/kg and 100 mg/kg for BHT in fats and oils for the professional manufacture of heat-treated foodstuffs, frying oil and frying fat, excluding olive pomace oil, fish oil, lard, beef, poultry and sheep fat. Dehydrated potatoes may only contain 25 mg/kg of gallates and BHA, individually or in combination, while chewing gums may contain 400 mg/kg of these antioxidants or BHT.

11.2.2.5 Health assessment

The ADI value for tocopherols is 0.15–2 mg/kg body weight. Vitamin E can cause several side effects in high concentrations that are not associated with the use of tocopherols as additives, but as vitamin supplements. A higher single dose of α-tocopheryl acetate (400–500 mg) could cause negative effects manifested by a higher intake of iodine by the thyroid gland. Dietary supplements containing amounts of about 1000 mg/day are therefore not recommended. No side effects are known to be caused by erythorbic acid and sodium erythorbate in the concentrations that are commonly used. The ADI value has not been established for 6-hexylresorcinol. There is negative evidence of carcinogenesis, and no significant untoward effects were observed in humans when it was used as an anthelmintic agent.

The toxicological evaluation of phenolic antioxidants is not yet entirely definitive. The only problem with the use of BHA (ADI = 0–0.5 mg/kg body weight, Group 2B agent possibly carcinogenic to humans, according to International Agency for Research on Cancer, IARC) seems to be the formation of lesions observed in experimental animals, although some pseudo-allergic reactions have also been reported. BHA in combination with high concentrations of vitamin C can produce free radicals, which can cause damage to the components of cells, including DNA. BHA is excreted in the urine after conjugation as D-glucuronide and sulfate; its oxidation is not significant. At high doses BHT (ADI = 0–0.125 mg/kg body weight, Group 3 agent, not classifiable as to its carcinogenicity to humans) can cause internal bleeding, which is related to its ability to reduce vitamin K. BHT can cause migraine in some people and liver damage in high concentrations; (pseudo)allergic symptoms have also been reported. BHT is metabolised primarily by oxidation of *tert*-butyl groups. TBHQ (ADI = 0–0.2 mg/kg body weight) is not allowed in some countries due to the lack of information on its toxicity (possible mutagenic activity). It is metabolised through oxidation and conjugation (sulfate and glucuronide). Gallates are metabolised after hydrolysis to the corresponding alcohols and gallic acid and methylation of gallic acid to 4-*O*-methylgallic acid. Gallic acid can cause eczema, stomach problems and hyperactivity. Gallates may cause contact dermatitis, which has been observed in bakers and in people handling gallates (ADI of propyl gallate = 0–2.5 mg/kg body weight).

11.3 Substances regulating odour and taste

Substances used for food flavouring are the most comprehensive group of additives. The following main groups are recognised:

- flavourings
- sweeteners
- acidulants and acidity regulators
- bitter substances and stimulants
- flavour enhancers.

11.3.1 Flavourings

Flavourings are substances that affect olfactory and gustatory receptors and induce human perception of smell or taste. They are used to give food taste and/or odour that would otherwise be absent or is not present in a characteristic intensity. Individual compounds responsible for the odour and taste of food raw materials and foods are described in detail in Sections 8.2 and 8.3, respectively.

11.3.1.1 Legislation

EU legislation defines different types of flavourings, such as:

- natural flavourings, that are derived from natural materials using physical, biotechnological (biochemical and microbiological) and other procedures;
- natural-identical, which are obtained by synthesis, but they are identical to substances present in natural materials;
- artificial flavouring substances, which are obtained by synthesis, but are not identical to natural flavouring substances.

In flavourings that are not chemically defined compounds, but mixtures of many substances, the following can be distinguished:

- flavouring preparations obtained by physical, enzymatic or microbiological processes from material of vegetable or animal origin or from other foodstuffs;
- process flavourings, which evolve flavour after heating and are obtained by heating a mixture of mostly amino acids and other nitrogen compounds with sugars or with other compounds for a maximum of 15 min;
- smoke flavourings, extracted from the waste products of the pyrolysis of materials used traditionally for smoked foods.

EU legislation also sets out general rules for the use of flavourings, requirements for labelling and maximum levels for substances, which raise concerns for human health. Flavourings are not substances, which exclusively have sweet, sour, bitter or salty tastes. Legislative regulations list more than 100 plant materials that are classified as foods or spices which, either in themselves or in the form of different products, can be used for food flavouring. Other flavouring substances are allowed in the production of tobacco products.

11.3.1.2 Health assessment

Substances which should not be added as such to food include (in alphabetical order) agaric acid, aloin, capsaicin, coumarin, hypericine, β-asarone, estragole, hydrogen cyanide, menthofuran, methyleugenol, pulegone, safrole, quassin, safrole, teucrin A, α-thujone and β-thujone. Table 11.5 lists the plant materials that contain these natural toxic substances. Examples of maximum levels of these substances naturally present in flavourings and food ingredients with flavouring properties, as well as their occurrence, health assessment and properties, are given in Chapters 9 and 10.

11.3.2 Sweeteners

Naturally occurring sweet substances are almost all monosaccharides (mainly glucose and fructose), disaccharides (sucrose and lactose) and sugar alcohols known as glycitols (such as D-glucitol, D-mannitol and xylitol) and other compounds. Nutritionists classify these sweet substances as dietary (nutritional) sweeteners because they are a source of energy and therefore have a certain nutritional value. With the exception of sugar alcohols, which are weakly cariogenic (D-glucitol and D-mannitol) or non-cariogenic (xylitol) and have little or no effect on blood glucose levels, mono- and disaccharides show cariogenic properties and their dietary intake is also associated with obesity and many other diseases.

Health, nutritional and economic aspects have led to the introduction of many natural, natural-identical and synthetic substances, which are generally characterised by having a higher sweetness than sucrose. Their physical, chemical and organoleptic properties are different from the properties of sugars, which may cause a number of food technology problems in formulation of novel products.

11.3.2.1 Classification

Sweeteners are classified according to their origin into the following groups:

- natural (e.g. thaumatin)
- natural-identical, which include synthetic substances identical to natural (such as sugar alcohols) or modified natural substances (such as neohesperidin dihydrochalcone)
- synthetic (such as saccharin).

From the nutritional point of view, two categories of sweeteners are recognised:

- nutritional (such as sugar alcohols)
- non-nutritional (virtually all other natural, modified natural and synthetic substances).

11.3.2.1.1 Nutritional sweeteners

Monosaccharides, disaccharides, sugar alcohols and other nutritional sweeteners derived from sugars are not considered food additives. These sweeteners are described in detail, including their relative sweetness, in Chapter 4, which deals with carbohydrates.

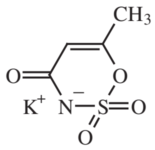
Sweet peptides (such as aspartame), proteins (e.g. thaumatin) and glycosides (stevioside), are a source of some energy, but in the quantities in which they are used, their contribution to the total energy intake is insignificant. These sweeteners are therefore ranked among non-nutritional sweeteners in Section 11.3.2.1.2.

Table 11.5 Main natural toxic compounds in plant materials used for aromatisation of foods and beverages.

Toxic compound	Plant English name	Plant Latin name
Agaric acid	White agaric mushroom	*Laricifomes officinalis*
Aloin	Aloe	*Aloe* spp.
β-Asarone	Calamus (sweet flag)	*Acorus calamus*
Coumarin	Woodruff	*Asperula odorata*
Coumarin	Yellow sweet clover (yellow melilot)	*Melilotus officinalis*
Coumarin	Cumaru	*Dipterix odorata*
Coumarin	Sweet grass	*Hierochloe odorata*
Estragole	Tarragon	*Artemisia dracunculus*
Hydrogen cyanide	Elder	*Sambucus nigra*
Hydrogen cyanide	Plum	*Prunus domestica*
Hypericin	St John's wort	*Hypericum perforatum*
Menthofuran	Peppermint	*Mentha piperita*
Methyleugenol	Clove	*Syzygium aromaticum*
Pulegone	Wrinkled-leaf mint	*Mentha crispa*
Pulegone	Peppermint	*Mentha piperita*
Pulegone	Pennyroyal	*Mentha pulegium*
Quassin	Quassia (bitterwood)	*Quassia amara*
Quinine	Cinchona trees (various species)	*Cinchona* spp.
Safrole and isosafrole	Nutmeg	*Myristica fragrans*
Teucrin A	Wall germander	*Teucrium chamaedrys*
α- and β-Thujone	Mugwort	*Artemisia vulgaris*
α- and β-Thujone	Wormwood	*Artemisia absinthium*
α- and β-Thujone	Santonica	*Artemisia cina*
α- and β-Thujone	Roman wormwood	*Artemisia pontica*
α- and β-Thujone	Tansy	*Tanacetum vulgare*
α- and β-Thujone	Common yarrow	*Achillea millefolium*
α- and β-Thujone	Musk Yarrow	*Achillea moschata*
α- and β-Thujone	Common sage	*Salvia officinalis*

11.3.2.1.2 Synthetic non-nutritional sweeteners

The relative sweetness of chemically modified natural and synthetic sweeteners is shown in Table 11.6.



11-25, acesulfame K

Acesulfame K

Acesulfame K (E950, K being the symbol for potassium) is a potassium salt of 6-methyl-1,2,3-oxa-3*H*-thiazin-4-one 2,2-dioxide (**11-25**). Some trade names are Sunett and Sweet One. This substance (approved in the EU in 1983 and in the USA in 2003 for general use, but not in meat or poultry) is often blended with other sweeteners (usually sucralose or aspartame) to mask its slightly bitter aftertaste. The stability of Acesulfame K makes it suitable for products

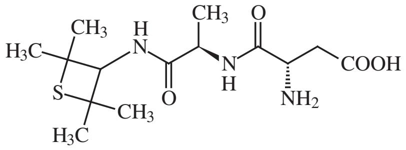
Table 11.6 Relative sweetness of chemically modified and synthetic sweet substances (sucrose = 1).

Compound	Sweetness
Modified substances	
Naringin dihydrochalcone	100–350
Neohesperidin dihydrochalcone	500–2000
(*E*)-Perillaldehyde oxime	350
Synthetic substances	
Acesulfame K	80–250
2-Amino-4-nitro-1-propoxybenzene	4000–5000
Alitame	2000
Aspartame	100–200
Cyclamates	30–60
Dulcin	70–350
Neotame	7000–13 000
Saccharin	200–700
Sucralose	600

that require a long shelf life (it decomposes at temperatures exceeding 235 °C). Acesulfame K is used in many applications and in particular in baked goods and in beverages. In carbonated drinks, it is almost always used in conjunction with another sweetener, such as aspartame or sucralose.

Alitame

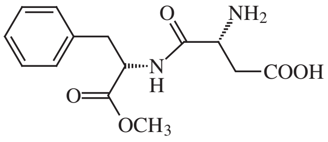
Like aspartame and neotame, alitame (in some countries known as aclame, **11-26**), L-α-aspartyl-*N*-(2,2,4,4-tetramethyl-3-thietanyl)-D-alanine amide, is an aspartic acid-based dipeptide, which is about ten times sweeter than aspartame and has no after-taste. As with aspartame, it is hydrolysed in acidic media, but does not contain bound phenylalanine and can therefore be used by people with phenylketonuria. Alitame is not approved for use in the EU or the USA, but is approved in Australia, China and some other countries.



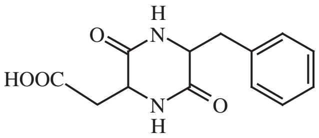
11-26, alitame

Aspartame

Aspartame (E951) is a methyl ester of linear dipeptide L-aspartyl-L-phenylalanine (**11-27**) that was used by several European Union countries in the 1980s, with EU-wide approval in 1994 (and in the United States in 1996 for general purpose). It does not show any after-taste. In non-aqueous media (such as powdered drinks, chewing gum and instant coffee), aspartame is stable. In acidic aqueous solution, depending on the pH and temperature, the ester bond of aspartame can be hydrolysed, creating the corresponding dipeptide (L-aspartyl-L-phenylalanine) and methanol. A product of the dehydration of this linear dipeptides is cyclic dipeptide cyclo-(L-aspartyl-L-phenylalanine, which is a derivative of 2,5-dioxopiperazine (**11-28**). On hydrolysis, dioxopiperazine provides a linear dipeptide, which is further hydrolysed to free aspartic acid and phenylalanine. Aspartame reactions are associated with a decrease in sweetness. Aspartame is therefore not suitable for all foods (especially acidic foods), or for all types of food processing. In non-alcoholic beverages, for example, about 20% or more of aspartame can be degraded at room temperature during four months of storage.



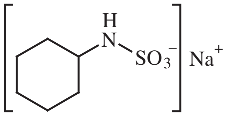
11-27, aspartame



11-28, 3-benzyl-6-carboxymethyl-2,5-dioxopiperazine

Cyclamates

Cyclamates is the generic name for cyclamic (cyclohexylsulfamic) acid and its sodium and calcium salts (E952), sodium cyclamate (**11-29**) and calcium cyclamate, which became popular in the 1950s. In 1958, cyclamate was classified as GRAS (Generally Recognized as Safe) in the United Staes, but it was subsequently banned in 1970 (see Section 11.3.2.3). Cyclamates are stable to heat, but exhibit minor after-taste; therefore they are used as sweeteners in baked goods, confectioneries, desserts, soft drinks, preserves and salad dressings, often in a mixture with saccharin (10 : 1) to produce a synergistic sweetening effect.

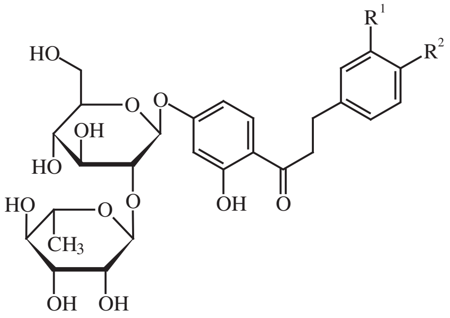


11-29, sodium cyclamate

Dihydrochalcones

A sweet taste is exhibited by some dihydrochalcones (see Section 9.4.2.6), which are obtained by the catalytic reduction of flavanone glycosides neohesperidin and naringin, which contain bound

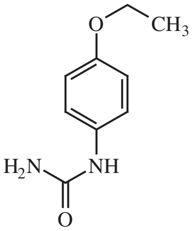
β-neohesperidose, α-L-Rha*p*-(1 → 2)-β-D-Glc*p* (**11-30**) in the C-7 position. Catalytic reduction of neohesperidin (hesperetin-7-neohesperidoside) obtained from bitter oranges yields neohesperidin dihydrochalcone (with β-neohesperidose bound to C-4), which has been approved in the EU as a sweetener since 1994 (E959). Naringin (naringenin-7-neohesperidoside) gives rise to naringin dihydrochalcone. Neohesperidin dihydrochalcone has some properties that limit its use as a sweetener. It has an intense cooling effect on the tongue, liquorice-like and bitter off-tastes that make it decidedly not sucrose-like and is slow in sweet taste onset. Because of these properties, neohesperidin dihydrochalcone is particularly suitable for use in the pharmaceutical industry and in animal nutrition. With respect to its properties as a sweetener and flavouring, neohesperidin dihydrochalcone has applications in sweets, canned vegetables, desserts, sauces and food supplements. Other important fields of neohesperidin dihydrochalcone application are the livestock industry, cosmetics (such as toothpastes) and pharmacy.



11-30, neohesperidin dihydrochalcone, R^1 = H, R^2 = OH
naringin dihydrochalcone, R^1 = OH, R^2 = CH_3

Dulcin

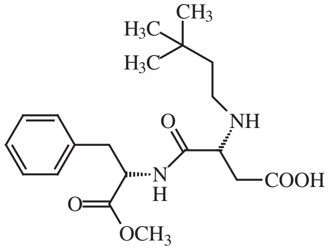
Dulcin (4-ethoxyphenylurea, **11-31**), also known as sucrol or valzin, was an important sweetener of the early 20th century and had an advantage over saccharin, with which in the past it had been used in blends. It has a more pleasant sweet taste and does not possess a bitter after-taste. However, in 1954, animal testing revealed unspecified carcinogenic properties of dulcin, which led to a ban on its use.



11-31, dulcin

Neotame

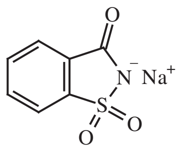
Neotame, *N*-(*N*-(3,3-dimethylbutyl)-L-α-aspartyl)-L-phenylalanine 1-methyl ester (**11-32**), is another sweetener from the family of dipeptides approved in the EU since 2011 as a flavour enhancer (E961) and since 2002 in the US. Neotame is a derivative of the dipeptide composed of aspartic acid and phenylalanine methylester, but unlike aspartame, aspartic acid occurs in the form of 3,3-dimethylbutylamide. Owing to the presence of the 3,3-dimethylbutyl group, peptidases are effectively blocked and the availability of phenylalanine is thus reduced, which is important for people suffering from phenylketonuria. Neotame functions effectively in a wide range of products, such as beverages, desserts, candy, ice creams, bakery goods and many others. It can be used alone or as part of a blend system with other non-nutritive or nutritive sweeteners.



11-32, neotame

Saccharin

Saccharin (E954) is a common name for the corresponding acid, 1,1-dioxo-1,2-benzothiazol-3-one, and its sodium (**11-33**), potassium and calcium salts. Substances in which the group $-SO_2-NH-$ is a part of rings are called sultames. It is the oldest sweetener, and became widespread during World War I (due to the shortage of sugar) and its popularity increased during the 1960s and 1970s among dieters. In the United States it was classified as GRAS. The onset of sweetness from saccharin is rapid. The disadvantage is that it shows faint metallic and bitter after-tastes, but they can be masked by lactose or saccharin may be used in combination with aspartame and other sweeteners. A 10 : 1 cyclamate : saccharin blend is common in countries where both these sweeteners are legal. Saccharin is very stable in foods even during heat treatments. The food industry uses saccharine salts in a variety of food and drinks, such as baked goods, breads and cookies, sweetened diet drinks, sweetened and fruit-flavoured yoghurt, jams, jellies and ice creams.

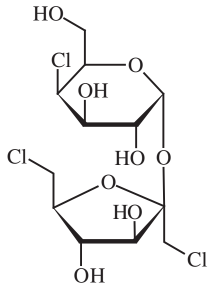


11-33, saccharin (sodium salt)

Sucralose

Sucralose (E955) or chlorogalactosaccharose is a trichloro derivative of a non-reducing disaccharide known as 4,1′,6′-trichloro-4,1′,6′-trideoxygalactosaccharose, or by the systematic

name 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside (**11-34**). In fact, it is a sucrose molecule in which three of the hydroxyl groups have been replaced by chlorine atoms. Sucralose is a relatively new sweetener, which has not yet been introduced in many countries. Sucralose was first approved for use in Canada in 1991, in 1999 as a general-purpose sweetener in the United States and in 2005 in all EU Member States. It appears to be only partially absorbed, which is followed by excretion in the kidneys. The sweet flavour profile is similar to that of sucrose, with a slightly bitter off-taste. Sucralose is resistant to acidic and enzymatic hydrolysis and is stable during thermal operations. At high temperature in aqueous solution, sucralose is hydrolysed to a small extent, producing 4-chloro-4-deoxy-D-galactose and 1,6-dichloro-1,6-dideoxy-D-fructose. Sucralose is widely used by the food, beverage and pharmaceutical industries and can be found in products, such as diet drinks, yoghurts and breakfast cereals.



11-34, sucralose

Other sweet compounds

In the past many other synthetic sweet substances were temporarily used as sweeteners, such as 2-amino-1-propoxy-4-nitrobenzene or 6-chlorotryptofan (about 100 times sweeter than sucrose), and so on. They have not found wider application, in particular due to toxicological reasons.

11.3.2.1.3 Natural non-nutritional sweeteners

Glycyrrhizin

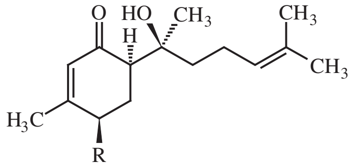
The rhizome of liquorice (*Glycyrrhiza glabra*, Fabaceae), native to Turkey, Iraq, Spain, Greece and northern China, contains triterpenoid saponins, the main sweet component of which, (with a characteristic liquorice taste, sometimes described as cooling is a glycoside known as glycyrrhizin (see Section 10.3.3.2.1). Glycyrrhizin extracted from liquorice rhizome is a mixture of potassium and calcium salts of glycyrrhizic acid. The aglycone is glycyrrhetic (glycyrrhetinic) acid. The sugar bound at C-3 is a disaccharide composed of two β-D-glucuronic acid units: β-D-Glc*p*A-(1 → 2)-β-D-Glc*p*A. The dried root of this plant has been used as a laxative and in confectionery. Glycyrrhizin is used to sweeten and flavour some candies, pharmaceutical preparations and tobacco products. It is reported to be relatively heat-stable. Glycyrrhizin is classified as a flavouring agent, although not as a sweetener. The relative sweetness of glycyrrhizin and some other natural sweet substances is shown in Table 11.7.

Table 11.7 Relative sweetness of natural sweet compounds (sucrose = 1).

Compound	Sweetness
Brazzein	2000
Dulcoside A	30
Glycyrrhizin	50
(+)-Hernandulcin	1250
Monellin	1500–3000
Osladin	3000
Perillaldehyde	12
Phyllodulcin	200–800
Rebaudioside A	130
Stevioside	100–300
Thaumatin	2000–3000

Hernandulcin

Sweet sesquiterpenoids (+)-hernandulcin, 6-(1,5-dimethyl-1-hydroxyhex-4-en-1-yl)-3-methylcyclohexen-2-one, and (+)-4β-hydroxyhernandulcin (**11-35**) occur at a level of about 0.04% in the sweet herb *Phyla dulcis* (syn. *Lippia dulcis*, Verbenaceae) native to tropical Central and South America. Leaves with the sweet taste (hernandulcin is about 1250 times sweeter than saccharose) are used in traditional medicine. Because of its slightly bitter taste and minty after-taste, hernandulcin has only limited use as a sweetener.



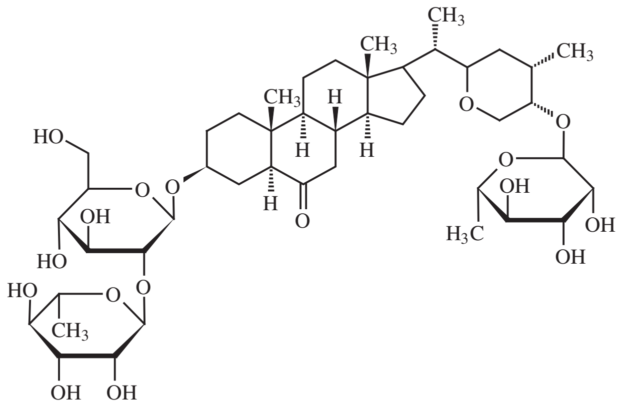
11-35, hernandulcin, R = H
4β-hydroxyhernandulcin, R = OH

Osladin

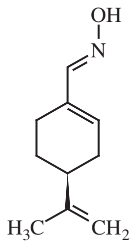
Osladin (**11-36**) is a steroidal saponin occurring in the rhizomes of the common polypody fern (*Polypodium vulgare*, Polypodiaceae). Sugar components are α-L-rhamnose and disaccharide α-L-Rha*p*-(1 → 2)-β-D-Glc*p* (β-neohesperidose). Osladin has not find applications due to toxicological reasons.

Perillaldehyde

The monoterpenic aldehyde (-)-perillaldehyde, also known as perillal (see Section 8.2.4.1.1), which occurs in small quantities as a



11-36, osladin



11-37, perillaloxime

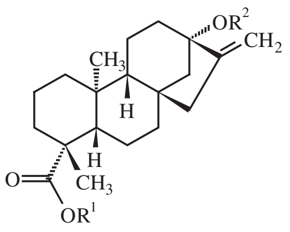
component of various essential oils (such as oils of citrus fruits), has a sweet taste. In the past, perillaldehyde was used as a starting compound for the synthesis of much sweeter (*E*)-perillaldehyde oxime (perillaloxime) called perillartin (**11-37**, Table 11.6).

Phyllodulcin

The isocoumarin phyllodulcin occurs in the leaves of big leaf hydrangea (*Hydrangea macrophylla*, Hydrangeaceae) native to Japan and in leaves of mountain hydrangea (*H. serrata*) native to Japan and Korea, along with related compounds (see Section 10.3.3.5.2). The sweetness of phyllodulcin is characterised by a slow onset and long-lasting sensation of sweet taste and an off-taste resembling liquorice. Phyllodulcin has found use in sweetening confectionery and chewing gums and to make regionally popular herbal teas, such as *amacha* tea in Japan, which is used in the celebration of Buddha's birth.

Stevioside

The sweet substance called stevioside is an *ent*-kaurene diterpene glycoside containing steviol (**11-38**) as the aglycone and β-D-glucose and disaccharide β-sophorose as sugar components. Stevioside is accompanied by other related glycosides, which include rebaudioside A, rebaudioside B, rebaudioside C, rebaudioside D, rebaudioside F, dulcoside A, rubusoside and steviolbioside (Table 11.8). Stevioside occurs in amounts of up to 6% in the leaves of stevia (*Stevia rebaudiana*, Asteraceae); a shrub native to tropical South America (Paraguay), the leaves of which can be used directly for sweetening, but the taste quality is not as good as that of isolated glycosides. Today, stevia is cultivated in the Far East (China, Japan, Korea and Malaysia). In Japan, it has been available to sweeten soft drinks, candy and chewing gums for decades. In some countries, health concerns have limited its availability; for



11-38, steviol ($R^1 = R^2 = H$)

Table 11.8 Structure of stevioside and related substances.

Trivial name	Substituents in steviol (see 11-38)	
	R^1	R^2
Stevioside	β-D-Glc*p*	β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside A	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside B	H	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside C (dulcoside B)	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-α-L-Rha*p*
Rebaudioside D	β-D-Glc*p*-(1→2)-β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside E	β-D-Glc*p*-(1→2)-β-D-Glc	β-D-Glc*p*-(1→2)-β-D-Glc*p*
Rebaudioside F	β-D-Glc*p*	β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→2)-β-D-Xyl*p*
Dulcoside A	β-D-Glc*p*	β-D-Glc*p*-(1→2)-α-L-Rha*p*
Rubusoside	β-D-Glc*p*	β-D-Glc*p*
Steviolbioside	H	β-D-Glc*p*-(1→2)-β-D-Glc*p* (β-soforosa)

example, the United States banned stevioside in the early 1990s (because of possible mutagenicity of steviol), but in 2008 it approved rebaudioside A as a food additive. Relatively recently (in 2011), stevioside was approved for use in the EU as steviol glycoside (E960), and maximum content levels for different types of foods and beverages were established.

11.3.2.1.4 Natural nutritional sweeteners

Natural nutritional sweeteners are proteins, but their potency is so high that, if used, they would not be significant sources of energy. All protein sweeteners have a slow onset of sweet taste and the sweetness lingers for some time. They are hydrolysed in acidic media, which results in the loss of the sweet taste. It is expected that sweet proteins are digested just as any other dietary proteins.

Brazzein

Brazzein is a sweet protein composed of 54 amino acids. It occurs in very sweet edible fruits of the plant *Pentadiplandra brazzeana* (Pentadiplandraceae), which is native to West Africa. Brazzein has a slight liquorice-like cooling effect in the mouth and is remarkably heat stable.

Miraculin

The glycoprotein miraculin occurs in small red berries of the West African shrub *Synsepalum dulcificum* (syn. *Richadella dulcifica*, Sapotaceae). Although it is itself tasteless, it adjusts the sour taste of acids to a sweet taste (e.g. taste of lemon juice), and this perception lasts about 1 h. Miraculin occurs as a tetramer (98.4 kDa), where a combination of two monomers forms two dimers linked by disulfide bridges. The relative molecular weight of the monomer is 24.6 kDa. Sugars bound in the glycoprotein (4 kDa, 13.9% of molar weight) are D-glucosamine (31%), D-mannose (30%), L-fucose (22%), D-xylose (10%) and D-galactose (7%). When heated over 100 °C, miraculin loses its taste-modifying property. Miraculin has no importance as a food additive (taste-modifying agent).

Monellin

Monellin is extracted from the fruit *Dioscoreophyllum volkensii* (syn. *D. cumminsii*, Menispermaceae) native to tropical African rainforests. Monellin is a sweet protein with liquorice-like flavour, which consists of two peptide chains, A and B, composed of a sequence of 45 and 50 amino acids, respectively (11.5 kDa). Under food processing conditions it is unstable and has no practical significance as a sweet substance.

Thaumatin

Thaumatin (also called thalin) is a mixture of sweet proteins extracted from the fruit of the tropical West African flowering plant *Thaumatococcus danielli* (Marantaceae), known as miracle fruit or miracle berry. It is the only protein sweetener approved in the EU (E957). The main components with sweet notes resembling liquorice are the proteins thaumatin I and thaumatin II, with a relative molecular weight of about 22 kDa (thaumatin I consists of 207 amino acids and contains one disulfide bond and no histidine). In addition to these proteins, the commercially available preparations contain several other sweet proteins (e.g. thaumatins a, b and c) and small amounts of polysaccharides (arabinogalactans and arabinoglucuronoxylans). Thaumatin is unstable with heat treatment. It is mainly used in food and drinks in combination with other sweeteners, for its flavour modifying properties and not as a sweetener, because it acts synergistically in combination with acesulfame K, saccharin, stevioside and other sweeteners.

11.3.2.2 Legislation

In the EU, most sweeteners are only permitted to be used in certain foods and are subject to specific quantitative limits (Table 11.9). Sorbitol (the correct name is glucitol, E420), mannitol (E421), maltitol (E965), lactitol (E966), xylitol (E967) and erythritol (E968) are approved as food sweeteners, and are described together with other sugar alcohols in Section 4.3.1.3.1. They are permitted to be used in *quantum satis* for confectionery, desserts and similar products. Common carbohydrates occurring in foods (glucose, fructose, sucrose and lactose) and honey are not considered sweeteners.

Sweeteners may be used to sweeten foods and for the preparation of table-top sweeteners, but are not intended to be processed for baby food. Foods and table-top sweeteners containing more

Table 11.9 List of current EU approved sweeteners and their E-numbers.

E-number	Name
E420	Sorbitol and sorbitol syrup
E421	Mannitol
E950	Acesulfame K
E951	Aspartame
E952	Cyclamic acid and its Na and Ca salts
E953	Isomalt
E954	Saccharin and its Na, K and Ca salts
E955	Sucralose
E957	Thaumatin
E959	Neohesperidin dihydrochalcone
E960	Steviol glycoside
E961	Neotame (as a flavour enhancer)
E962	Salt of aspartame-acesulfame
E965	Maltitol and maltitol syrup
E966	Lactitol
E967	Xylitol
E968	Erythritol

Table 11.10 Quantitative limits and applications for synthetic non-nutritional sweeteners.

Maximum usable dose (mg/kg or mg/l)	Sweetener[a]						
	Ac	As	Cy	Nc	Sa	Su	Th
Non-alcoholic beverages	350	600	250	30–50	80–100	200–300	-
Desserts and similar products	350	500–1000	250	50	100	400	-
Confectionery with no added sugar	500	1000	500	100	500	1000	50
Energy reduced james, jellies and marmalades	1000	1000	1000	50	200	400	-
Chewing gums with no added sugar	2000	5500	1500	400	1200	3000	50
Cider and perry	350	600	-	20	80	50	-
Beer (various types)	350	600	-	10	80	250	-

[a] Ac = acesulfame K, As = aspartame, Cy = cyclamates, Nc = neohesperidine chalcone, Sa = saccharin, Su = sucralose, Th = thaumatin.

than 10% sugar alcohols must carry a warning on the packaging that excessive consumption may have a laxative effect. Top-table sweeteners and foods containing aspartame must be labelled that they contain a source of phenylalanine. Table 11.10 provides the maximum usable doses of approved sweeteners in some selected foodstuffs.

11.3.2.3 Health assessment

The ADI value was not specified for sugar alcohols derived from monosaccharides (xylitol, glucitol and mannitol) and disaccharides (maltitol, isomaltitol and lactitol).

Acesulfame K does not exhibit mutagenic or other toxic effects. It is rapidly absorbed and excreted mainly in the urine as an unchanged compound. In certain countries (including EU countries), acesulfame K has many uses, but in others it is only used for toothpastes and mouthwashes. The ADI value is 15 mg/kg body weight for children and adults. Acute toxicity in humans may manifest itself by headaches.

Aspartame has been the most controversial artificial sweetener by far because of its potential toxicity. It is hydrolysed to phenylalanine, aspartic acid and methanol and partially metabolised to glutamate. The daily intake of phenylalanine from food, which is estimated at 3.6 g, remains virtually unchanged using aspartame, but in people with impaired phenylalanine metabolism (phenylketonuria) some problems could arise. In some foods, where up to 5% of aspartame may be transformed into 2,5-dioxopiperazine, there has been some doubt about its possible carcinogenic properties. The toxicological data suggest that normal consumption of aspartame is not risky (ADI = 40 mg/kg body weight for aspartame and 7.5 mg/kg body weight for the corresponding 2,5-dioxopiperazine). Acute toxicity may be manifested by headaches, dry mouth, dizziness, mood change, nausea, vomiting, reduced seizure threshold and thrombocytopaenia (an abnormally low number of platelets in the bloodstream).

Cyclamates are partially absorbed in the digestive tract (individually within 1–60%) and the rest is then transformed by intestinal bacteria into cyclohexylamine. Cyclohexanol and cyclohexane are produced in small amounts. Problems arose with cyclamates in 1969, when a single scientific study showed bladder carcinogenicity in rats fed with cyclamates due to the formation of cyclohexylamine, which led to an immediate ban on use of the compounds in many countries. More than two dozen other studies on its safety reportedly failed to show the same results, which has led to re-approval of the use of cyclamates in many countries, including the EU, although they remain banned in the United States (ADI = 11 mg/kg body weight).

Ingested neohesperidin dihydrochalcone is hydrolysed in the same way as other related, naturally occurring flavonoids, yielding sugars and the corresponding aglycone. The aglycone is partly absorbed, metabolised, conjugated and excreted via bile and urine, partly undergoes bacterial ring cleavage (of the C-ring) and subsequently cleavage of the three-carbon bridge. It has been shown that neohesperidin dihydrochalcone can produce some side effects, such as nausea and migraine, at concentrations of around 20 mg/kg and above, but this effect is not well documented (ADI => 5000 mg/kg body weight).

Neotame is recognised as safe and, in toxicological studies, no adverse findings related to neotame were found in clinical observations in test animals. At high doses, acute toxicity may be manifested by headache and hepatotoxicity. The ADI for neotame was set at 2 mg/kg body weight by JECFA (the Joint FAO/WHO Expert Committee on Food Additives) in 2003.

Saccharine is slowly but almost completely absorbed in the digestive tract and is rapidly excreted unchanged in the urine. The unabsorbed portion is excreted in faeces. Some doubts persist about its potential carcinogenicity or co-carcinogenicity (ADI = 5 mg/kg body weight), as saccharin has been shown to produce urinary bladder tumours in rats. This finding has led to a ban in some countries and a proposed ban in the United States, but this phenomenon has not been observed in mice or in any other species, including humans. Acute toxicity may be manifested by nausea, vomiting and diarrhoea.

Sucralose consumption does not have adverse health effects at doses up to 10 mg/kg body weight and repeated doses of up to 5 mg/kg per day for several weeks. The acute toxicity of sucralose

may be manifested by migraine in sensitive individuals and by diarrhoea. The ADI for sucralose was set at 15 mg/kg body weight.

Stevioside is not degraded in the stomach juice and its uptake by the gastrointestinal tract is very low. All the stevioside reaching the colon is degraded by microorganisms into steviol, the only metabolite found in faeces. In urine, no stevioside or free steviol are present, except small amounts of steviol glucuronide. Studies of the toxicity of stevioside found no clinical signs of toxicity or morphological or histopathological changes in test animals. A European Food Safety Authority (EFSA) panel established an ADI for steviol glycosides, expressed as steviol equivalents, of 4 mg/kg body weight.

Thaumatin is not absorbed as such, but is completely hydrolysed in the digestive tract to amino acids (about as fast as egg albumin). Thaumatin is not mutagenic, teratogenic or allergenic and its consumption does not pose any health risk (there is currently no listed ADI for thaumatin).

In the United States, glycyrrhizin is generally recognised as safe. The European Union suggests that an intake of 100 mg/day (equivalent to approximately 50 g of liquorice sweets) would be unlikely to cause adverse effects (pseudohyperaldosteronism leading to hypocalcaemia alkalosis associated with a low serum potassium level and lower aldosterone secretion) in the majority of adults. People suffering from hypertension should avoid excessive consumption of liquorice products. The ADI for glycyrrhizin is suggested at 0.2 mg/kg/ body weight.

11.3.3 Acidulants and acidity regulators

Acidulants used as food additives are inorganic and organic acids generally identical to those that occur naturally in foods. Acids are most commonly used for their sour taste (see Section 8.3.3). They often also have other beneficial properties. Some acids:

- exhibit antimicrobial effects and are therefore used simultaneously as preservatives, such as vinegar– acetic acid (E260), propionic acid (E280) and other acids;
- have significant organoleptic properties (taste and smell) and are used as flavouring agents, for example actic acid (E260), succinic acid (E363), fumaric acid (E297), adipic acid (E355), lactic acid (E270), citric acid (E330) and malic acid (E296);
- act as colour stabilisers, such as ascorbic acid (E300) in meat products and citric acid (E330) in fruit products;
- act as sequestrants and synergists of antioxidants, for example calcium disodium salt of ethylenediaminetetraacetic acid (E385), citric (E330), tartaric (E334), malic (E296), ascorbic (E300) and phosphoric acids (E338);
- are substances that modify the texture, for example citric acid (E330) allows the formation of some pectin gels, milk clotting by chymosin and inhibits the formation of crystals in confectionery;
- suppress the formation of hazes, such as lactic acid (E270) in brine of fermented olives;
- are agents that hydrolyse proteins, such hydrochloric acid (E507) in the production of acidic protein hydrolysates.

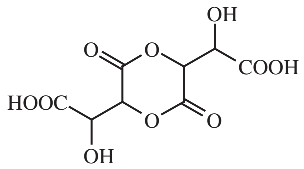
Acidifying agents also include substances that produce acids by hydrolysis or during heating. This group of additives includes salts used as raising agents, such as sodium (E500), potassium (501) and ammonium carbonates (E503) releasing carbon dioxide in the dough. These additives are also used in the production of sparkling beverages. In durable fermented salami, dairy and other products, δ-lactone of D-gluconic acid (D-glucono-1,5-lactone, E575) is used, from which D-gluconic acid arises by hydrolysis (see Section 4.3.2.1).

Acidity regulators or pH regulators maintain the acidity and alkalinity of foods. They mostly include salts of various acids with buffering effects and alkaline agents. For example, dispersion stabilisers for dairy products (melting salts) and meat products (substances that increase meat water holding capacity) are mostly various di-, tri- and polyphosphates (E339, E400, E411, E450–E452). Sodium hydrogen carbonate (E500) is used for pH adjustment in the manufacture of dark, Dutch-processed cocoa powder to neutralise its natural acidity. Sodium carbonate (E500) or sodium hydroxide (E524) are used for neutralisation of acidic protein hydrolysates, while sodium hydroxide (E524) is employed for olive debittering (see Section 8.3.5.2) and for peeling fruits and vegetables.

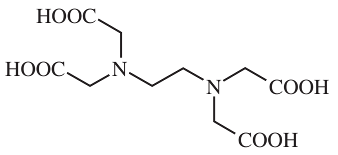
11.3.3.1 Legislation

Many substances may be used in food production only in the amount necessary to achieve the desired effect. Phosphates or mixtures thereof may be only used for listed foods, and their amount is limited.

For example, phosphoric acid (E338) is allowed for flavoured soft drinks (such as cola drinks) in a quantity of up to 700 mg/l, phosphates (as P_2O_5) for soft fresh cheeses (2000 mg/kg), melted cheese (melting salt, 20 000 mg/kg), meat products (for the increase of water binding capacity, 5000 mg/kg) and powder whiteners for beverages (30 000 mg/kg). The lactide metatartaric acid (E353, **11-39**), prepared by heating tartaric acid, is allowed only for wines (100 mg/l), calcium disodium salt of ethylenediaminetetraacetic acid (E385, **11-40**) at a concentration of 75 mg/kg for emulsified sauces, mayonnaises, pickled products from crustaceans, molluscs and fish and up to 250 mg/kg for canned legumes, artichokes and mushrooms. To highlight the colour of black olives, ferrous salts of some organic acids are allowed, lactate (E579) or gluconate (E579 at a level of 150 mg/kg (calculated as iron) that form black complexes



11-39, metatartaric acid



11-40, ethylenediaminetetraacetic acid

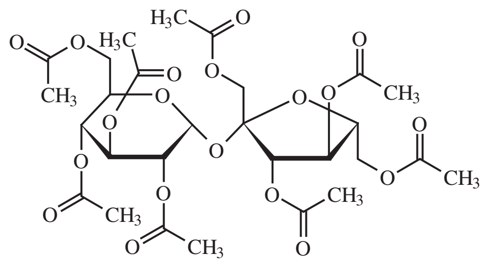
with oxidised fruit polyphenols. Urea (E927) may only be used in food supplements (200 mg/kg), and triethyl citrate only for egg whites (and only in the specified quantity).

11.3.3.2 Health assessment

Acids and their salts are generally considered to be natural food constituents. Citric, fumaric and succinic acid are intermediates of the citric acid cycle, and propionic acid is metabolised like other fatty acids (ADI values are not given). There are, however, reservations over the use of certain acids. Acetic acid induces epidermal reactions and other allergic type symptoms in susceptible individuals. Adipic acid (ADI = 5 mg/kg body weight) may affect the growth of animals. Restrictions also relate to fumaric acid (ADI = 6 mg/kg body weight), racemic malic acid (ADI = 100 mg/kg body weight, but L-isomer is a normal food constituent) and L-tartaric acid (ADI = 30 mg/kg body weight). Racemic and D-lactic acid may cause acidosis in infants, vomiting and dehydration. The ADI value is not set, but it is not recommended to use these acids in infant nutrition.

11.3.4 Bitter substances and stimulants

A large number of organic and inorganic compounds have a bitter taste, and these are discussed in other contexts in Section 8.4.5.1. Bitter tasting substances derived from plant materials (such as hops, wormwood and other herbs), which are used for aromatisation are classified as flavourings, as well as the category of bitter tasting additive substances and stimulants to which the alkaloids caffeine (see Section 10.3.3.1.8) and quinine (see Section 10.3.3.1.5) belong. None of these compounds have E-numbers. Another bitter additive substance is octaacetylsaccharose, better known as sucrose octaacetate (**11-41**). Pesticide products containing sucrose octaacetate as an inert ingredient are used as insect repellents, herbicides, flea and tick sprays and other insecticides. Other commercial uses of sucrose octaacetate include impregnating and insulating papers, as well as in lacquers and plastics.



11-41, octaacetylsaccharose

11.3.4.1 Legislation

In the production of food, caffeine and quinine may be used directly or as components of flavourings. Their amount was limited in the past, but according to new legislation (see Section 10.3.3.1.8) only beverages that contain caffeine concentration higher than 150 mg/l must be labelled as having 'High caffeine content'. The maximum content of quinine in bitter drinks is not limited either. Sucrose octaacetate has been approved by the FDA as a food additive in the US, but not in the EU, where it may be added to foods only in the specified quantities.

11.3.4.2 Health assessment

In the gastrointestinal tract, octaacetylsaccharose is hydrolysed to acetic acid and sucrose, which are metabolised in the normal way. The health evaluation of caffeine and quinine has been described elsewhere (see Sections 10.3.3.1.8 and 10.3.3.1.5, respectively).

11.3.5 Flavour enhancers

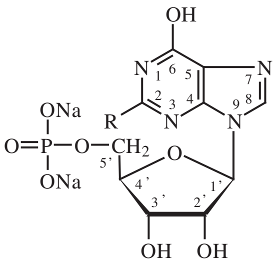
Flavour enhancers are substances that intensify or modify the original flavour of certain foods, even though they do not have their own flavours. Additives of this category include:

- L-glutamic acid (E620) and its salts, sodium hydrogen glutamate (E621), potassium hydrogen glutamate (E622), ammonium hydrogen glutamate (E624), calcium glutamate (E623) and magnesium glutamate (E625);
- purine 5′-nucleotides, which include inosine 5′-phosphate (inosinic acid, IMP, E630), disodium (E631), dipotassium (E632) and calcium inosinate (E633), guanosine 5′-phosphate (guanidylic or guanylic acid, GMP, E626), disodium (E627), dipotassium (E628) and calcium guanylate (E629).

Of particular importance are glutamic acid and sodium hydrogen glutamate. This salt is the active form that shows the taste referrd to as umami (see Section 8.3.4). At concentrations of 0.05–0.8%, in which it is added as an additive, it amplifies and enhances the flavour of meat and vegetable products, such as soups and sauces, meat and vegetable preserves, tomato juice, ketchup, mayonnaise and other products.

Synergistic effects to the taste of glutamate are shown by disodium 5′-ribonucleotides: IMP and GMP (**11-42**), which are added to foods in amounts ranging from 0.001 to 0.2%. When used, the amount of glutamate can be reduced about ten times, while maintaining the same intense umami taste. Commonly used is a mixture containing 95% w/w sodium hydrogen glutamate, 2.5% w/w IMP and 2.5% w/w GMP.

Detection thresholds of IMP and GMP are 250 and 125 mg/l, respectively. The detection threshold of a mixture of these substances (in weight ratio 1 : 1) is 63 mg/l, in combination with glutamate (at a concentration of 8 g/l; its detection threshold is 120 mg/l) it is as low as 0.31 mg/l. All three compounds are common food ingredients (Table 11.11). In some products, however,



11-42, 5′-nucleotides
5′-IMP, R = H
5′-GMP, R = NH_2
5′-XMP, R = OH

Table 11.11 Natural content of glutamic, inosinic and guanylic acids in selected foods.

Food	Content (mg/kg)		
	Free Glu	IMP	GMP
Pork meat	230	1860	37
Chicken meat	440	1150	22
Peas	750	0	0
Tomatoes	2 460	0	0

the glutamate content is very high (e.g. about 40 g/kg in acid protein hydrolysates, which corresponds to 100 g/kg of dry matter). In meat and fish IMP prevails, as it arises from ATP via deamination of AMP *post mortem* (Figure 11.12). IMP is then degraded via inosine, hypoxanthine and xanthine to uric acid. The amount of IMP in meat extracts (highly concentrated meat stocks) used in cooking reaches about 10 g/kg. In the meat of crustaceans, the main nucleotide is adenosine 5′-phosphate (AMP). Some mushrooms and yeast extracts used as food additives (flavourings) and as nutrients for bacterial culture media in microbiology have a higher content of GMP. Xanthosine 5′-phosphate (xanthylic acid, XMP) has similar properties as IMP and GMP.

11.3.5.1 Legislation

Glutamic, inosinic and guanylic acids and their sodium and potassium salts can be used as flavour enhancers, individually or in combination, up to the maximum allowable amount. The permissible amount of glutamic acid in foodstuffs in general (excluding soft drinks) is 10 g/kg. Specified amounts of this amino acid and nucleotides are prescribed for condiment preparations.

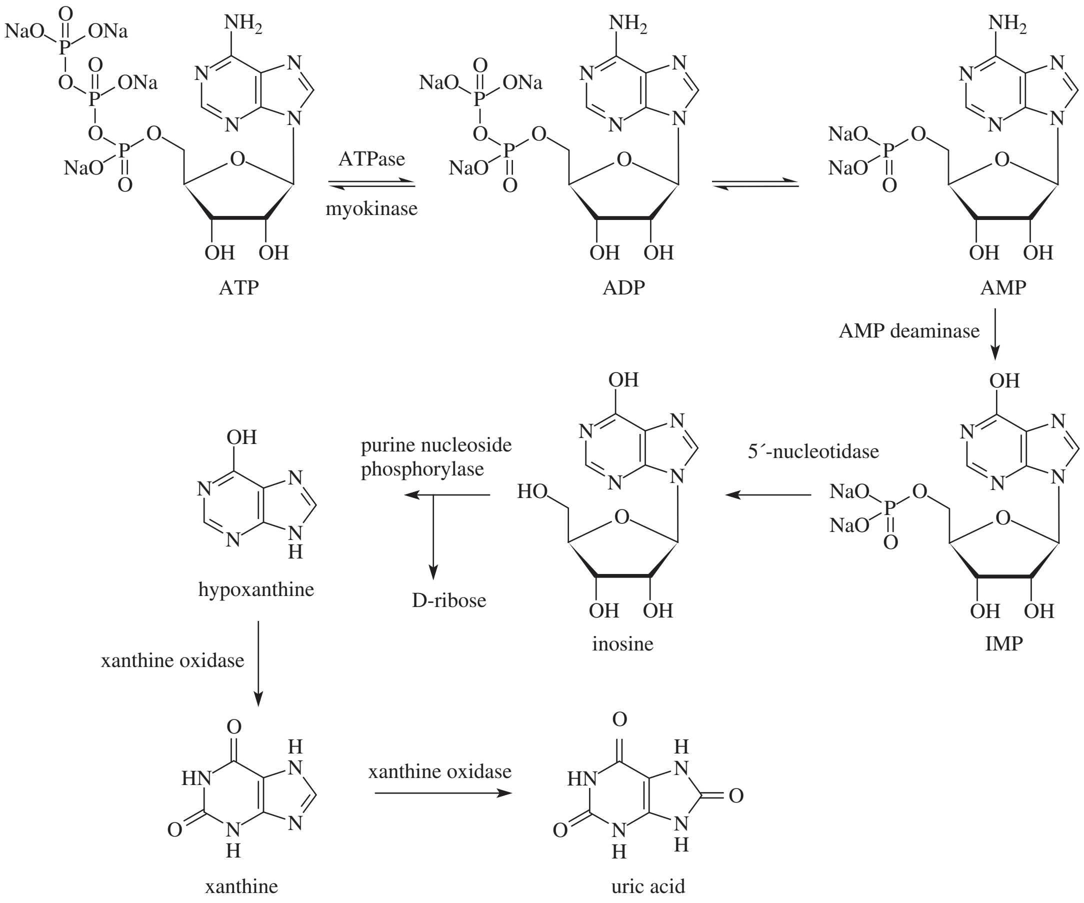


Figure 11.12 Formation and degradation of IMP in meat.

Table 11.12 List of current EU approved natural and natural-identical colours and their E-numbers.

E-number	Name	E-number	Name
E100	Curcumin	E160a	Carotenes
E101	Riboflavin and riboflavin 5′-phosphate	E160b	Annatto, bixin and norbixin
E120	Cochineal, carminic acid and carmines	E160c	Paprika extract, capsanthin, capsorubin
E140	Chlorophylls and chlorophyllins	E160d	Lycopene
E141	Copper complexes of chlorophyll, chlorophyllins	E160e	β-Apo-8′-carotenal
E150a	Plain caramel	E160f	Ethyl β-apo-8′-carotenoate
E150b	Caustic sulfite caramel	E161b	Lutein
E150c	Ammonia caramel	E161g	Canthaxanthin
E150d	Sulfite ammonia caramel	E162	Beetroot Red, betanin
E153	Vegetable carbon	E163	Anthocyanins

11.3.5.2 Health assessment

In the past there have been some reservations over excessive intake of glutamic acid and its salts, because it was associated with the so-called Chinese restaurant syndrome manifested in sensitive individuals by headaches, anxiety, digestive problems and burning in the upper parts of the body, but these side effects have not been scientifically proven. When glutamic acid or glutamates are added to foods, legislation requires that it be listed on the label (see Section 8.3.4). Many countries have restricted the amount of glutamic acid, glutamate and 5′-nucleotides added to foods legislatively. In some countries, however, these substances are not regulated at all (e.g. in Japan). The ADI for sodium hydrogen glutamate is 120 mg/kg body weight, ADI values for 5′-nucleotides are not specified, but salts of IMP (inosinates) may not be used in products intended for children under 12 weeks. As 5′-nucleotides are metabolised to uric acid, they should be avoided by people suffering from the form of arthritis known as gout. However, the concentrations used are generally so low that no effects are to be expected. Asthmatic people should likewise avoid these substances.

11.4 Substances modifying colour

Stabilisation of natural food colours and colouring of food has been carried out since time immemorial for aesthetic reasons, to make food more visually appealing, but the physiological reasons are also significant. Colours are used to restore the original appearance of food, whose pigments have been affected by processing, storage, packaging and distribution, and to give colour to food that would otherwise be colourless, whereby its visual acceptability may be impaired. Another reason is the standardisation of colour, for example the compensation of seasonal fluctuations. The attractive colour of food is related to its likeability, increases the secretion of gastric juices in the consumer and leads to better utility of the food. In some cases, the natural colour may be unwanted and can be removed using bleaching agents.

11.4.1 Colours

11.4.1.1 Classification

Pigments found in foods are divided according to their origin into:

- natural pigments
- natural-identical synthetic pigments
- synthetic dyes.

11.4.1.2 Natural colours

Natural and natural-identical synthetic pigments approved for colouring food are described in detail in Chapter 9 and caramel colours, one of the oldest and most widely-used food colourings, in Section 4.7.6. They are listed in Table 11.12.

11.4.1.3 Synthetic dyes

11.4.1.3.1 Structure and nomenclature

Synthetic dyes generally have more intense colour than natural dyes, are more stable and do not introduce any characteristic odours and tastes into coloured food. Therefore, synthetic dyes are widely used in food practice, mainly for practical and economic reasons. Food colours are contained in many foods, including snack foods, margarine, cheese, jams and jellies, and desserts, drinks and other products.

The following dye classes are recognised according to structure:

- azo dyes (monoazo, bisazo-, trisazo-to polyazo dyes)
- diphenylmethane and triphenylmethane dyes
- pyrazolone dyes

- nitro dyes
- xanthene dyes
- anthraquinone dyes
- quinoline dyes
- indigo dyes.

Other dyes, such as acridine, diazonium, phthalocyanin, tetrazolium and thiazole dyes, are not approved for foods. According to their physico-chemical properties, synthetic dyes may be classified into:

- sour dyes
- alkaline dyes
- neutral dyes.

According to their solubility, synthetic dyes are divided into:

- lyophylic dyes (soluble in water)
- lipophilic dyes (soluble in fats).

The list of synthetic dyes approved in the EU is shown in Table 11.13. All synthetic dyes approved as food dyes are

Table 11.13 Synthetic EU approved food dyes, their E-numbers and some attributes.

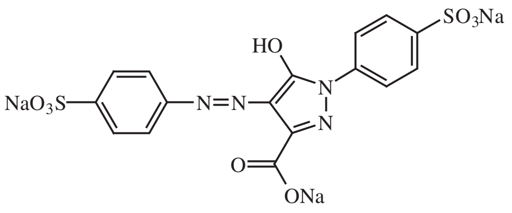
E-number	Name	Other names[a]	Class	Colour
E102	Tartrazine	Acid yellow T, Hydrazine yellow, FD&C Yellow No:5; C.I. Acid Yellow 23	Monoazo	Yellow
E104	Quinoline yellow	Food Yellow-13, D&C Yellow No:10, C.I. Acid Yellow-3	Quinoline	Yellow
E110	Sunset Yellow FCF, Orange Yellow S	FD&C Yellow No:6, C.I. Food yellow 3	Monoazo	Orange
E122	Azorubine, Carmoisine		Monoazo	Blue-red
E123	Amaranth	FD&C Red No. 2, C.I. Acid Red 27, C.I. Food red 9	Monoazo	Blue-red
E124	Ponceau 4R, Cochineal Red A		Monoazo	Red
E127	Erythrosine	FD&C Red No:3	Xanthene	Red
E129	Allura Red AC	FD&C Red No:40	Monoazo	Blue-red
E131	Patent Blue V		Monoazo	Red
E132	Indigotine, Indigo carmine[b]	FD&C Blue No:2	Triphenylmethane	Green-blue
E133	Brilliant Blue FCF	FD&C Blue Dye No:1, C.I. Acid blue 9, C.I. Food blue 2, C.I. Pigment blue 24	Indigo	Dark blue
E142	Green S	C.I. Acid green 50, C.I. Food green 4	Triphenylmethane	Green-blue
E151	Brilliant Black BN, Black PN	C.I. Food Black 1	Triphenylmethane	Green
E154	Brown FK		Bisazo	Black
	Brown HT		Monoazo, bisazo and trisazo	Brown
E180	Litholrubine BK		Bisazo	Brown

[a] FD&C = FD&C numbers indicate that the US Food and Drug Administration (FDA) has approved the colorant for use in Foods, Drugs and Cosmetics). Colorants without FD&C numbers are banned in the United States. Colour additives FD&C Green No. 3 (for general use), Orange B (for casings or surfaces of frankfurters and sausages) and Citrus Red No. 2 (for skins of oranges not intended or used for processing) are approved in the United State, but are not approved in the EU. C.I. = C.I. (Colour Index) number.

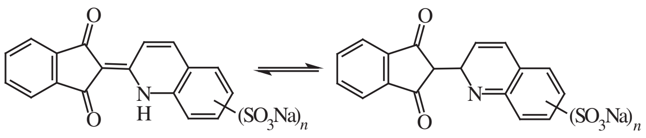
[b] Indigotine occurs as a natural pigment (in shrub *Indigofera tinctoria*), though commercially it is produced synthetically.

water-soluble compounds. The most represented are acidic dyes containing sulfonic groups, carboxyl groups and hydroxyl groups. Most of them belong to the azo dyes; some are di- and triphenylmethane dyes, nitro dyes and xanthene dyes. Basic dyes contain one or more free or substituted amino groups. These include the majority of di- and triphenylmethane dyes and certain azo dyes. All dyes are used in the form of salts (usually sodium, potassium or calcium salts).

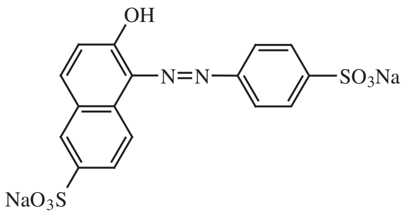
Specific properties of dyes depend on the functional groups present. A characteristic is the presence of two types of functional groups, **chromophores** and **auxochromes**. Chromophore groups are related to the class of dyes (such as azo dyes and nitro dyes) and are responsible for the behaviour of dyes in oxidation and reduction reactions. Auxochrome groups are responsible for the staining properties and behaviour to acids, alkalis, light and heat. The structures of synthetic dyes approved in the EU are given in formulae **11-43** to **11-58**.



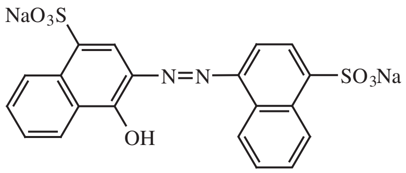
11-43, Tartrazine



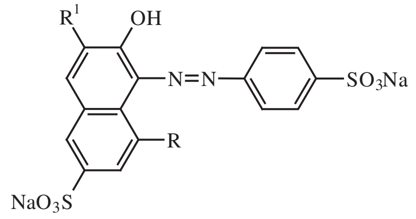
11-44, Quinoline Yellow (n = 1–3)



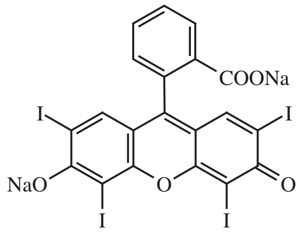
11-45, Sunset Yellow FCF



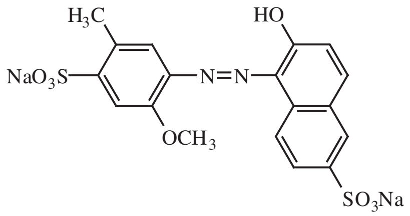
11-46, Azorubine



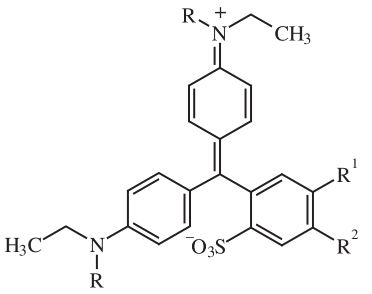
11-47, Amaranth, $R^1 = SO_3Na$, $R^2 = H$
Ponceau 4R,$R^1 = H$, $R^2 = SO_3Na$



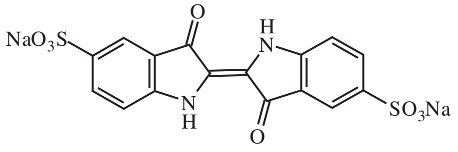
11-48, Erythrosine



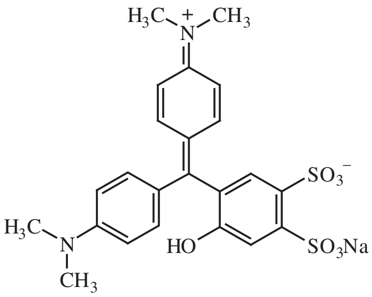
11-49, Allura Red AC



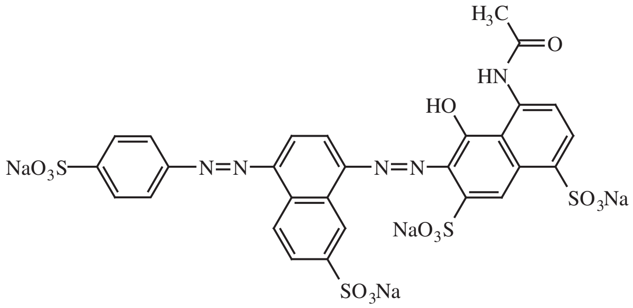
11-50, Patent Blue V, $R = CH_2CH_3$, $R^1 = OH$, $R^2 = SO_3Na$
Brilliant Blue FCF, $R^1 = H$, $R^2 = H$



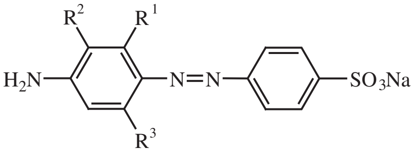
11-51, Indigotine



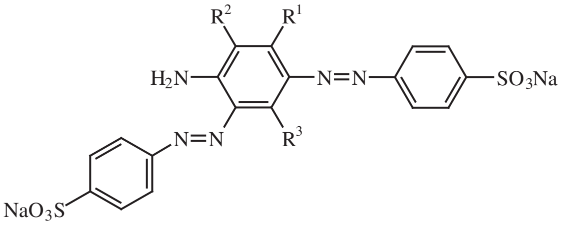
11-52, Green S



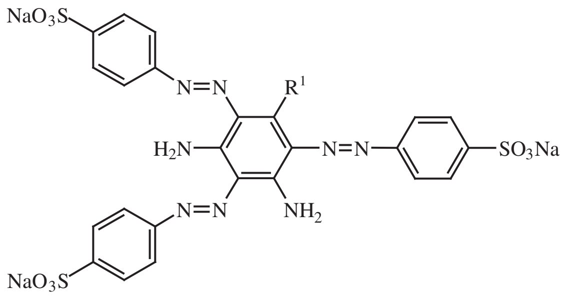
11-53, Brilliant Black BN



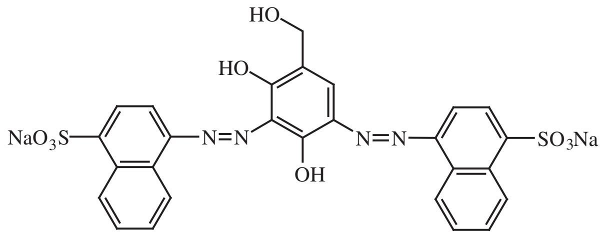
11-54, Brown FK (monoazo components, $R^1 = H$ or NH_2, $R^2 = H$ or CH_3, $R^3 = H$ or NH_2)



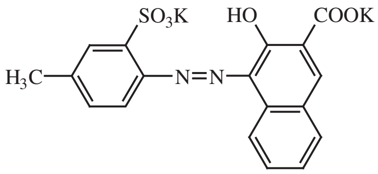
11-55, Brown FK didiazo components, substituents: see monoazo components



11-56, Brown FK (triazo components, substituents: see monoazo components)



11-57, Brown HT



11-58, Lithorubine BK

11.4.1.3.2 Properties and applications

In addition to toxicological criteria, it is required that synthetic dyes have to be chemically pure substances, which do not influence any other organoleptic properties of food (with the exception of colour). They must be stable to changes of pH and on exposure to light. Generally, there is no such dye that is suitable for all applications and situations, therefore the dye used is usually composed of several components and represents a mixture of dyes.

The majority of synthetic dyes have sufficient stability, particularly in dry foods and foods protected from light. Their stability is also sufficient under normal conditions of production, processing and during storage of foods. Azo dyes can relatively easily reduce metal ions and certain reducing agents (such as sulfur dioxide or ascorbic acid present in beverage) yielding colourless products. Triphenylmethane, indigo and xanthene dyes are more stable, but due to UV radiation Indigotine and Erythrosine may be decolourised.

Synthetic dyes are available:

- in the form of dispersions, pastes, aqueous or non-aqueous solutions (mainly in propylene glycol or glycerol) or in the solid state (such as water-soluble granules or powders);

- in the form of water-soluble lake pigments (commonly known as lakes).

Solid products are especially suitable for colouring beverages and emulsions and pastes for colouring confectionery and bakery products, while liquid paints are used for colouring dairy products. Lake pigments are obtained by adsorption of dyes on hydrated alumina. They have different content of dyes, normally 10–40%. The minimum content of dyes in lakes is not prescribed, as the colour is related to the technology of their production, which determines their colouring power, shade and dispersibility. Lakes are not oil soluble, but are oil dispersible. Lakes that contain individual dyes or mixtures thereof are delivered in solid form or as

dispersions in hydrogenated vegetable oils, propylene glycol, and glycerol or sugar syrup. These forms of food colours are more stable than dyes and are ideal for colouring products containing fats and oils or items lacking sufficient moisture to dissolve dyes.

Some dyes, such as azo dyes Amaranth and SunsetYellow FCF, can react in slightly acidic media with bisulfites (sulfur dioxide) to produce coloured products (e.g. a yellow product arises from Sunset Yellow FCF), but some dyes, such as Tartrazine and Azorubine, yield colourless products. The former group of azo dyes have hydroxyl groups in the *ortho*-position to the azo bond as well as unsubstituted *para*-positions in the naphthalene nucleus. Such dyes exist predominantly as hydrazone tautomers rather than strictly azo compounds, which facilitates the addition of bisulfite ion to the *para*-position (Figure 11.13). With Azorubine, the addition of bisulfite ion at the *para*-position appears not to take place, probably as a result of charge delocalisation via the fused aromatic ring of the naphthalene system. It is probable that the dye reacts with bisulfite to form an unstable complex that hydrolyses to a colourless hydrazo product (Figure 11.14).

As with sulfur dioxide, the reaction of ascorbic acid with azo dyes frequently used in combination, for example in fruit drinks, leads to dye degradation. The stability of the most frequently used dyes (at pH 3.0 and 4.0) in the presence of ascorbic acid decreases as follows: Tartrazine > Sunset Yellow FCF = Amaranth, Ponceau 4R > Azorubine > Brilliant Black BN.

11.4.1.4 Inorganic pigments

Inorganic compounds in food are only used in special cases. For example, for the surface treatment and decoration of dragees, candies and similar products, the white pigments calcium carbonate (E170) and titanium dioxide (E171), red, yellow or black iron oxides and hydroxides (E172) and aluminum (173), silver (E174) or gold (E175) pigments are used. Silver and gold pigments are similarly used for decoration of specialty liqueurs (in specified quantities).

11.4.1.5 Legislation

Flavour-active materials with simultaneous colouring effect (such as paprika, saffron and turmeric) are not considered colorants, nor are colorants intended to colour the inedible external parts of foods (such as paraffin wax coatings of cheeses and sausage casings).

Some dyes may only be used for certain purposes and to the maximum amount allowed. For example, Amaranth is allowed for aperitif wines and spirits (30 mg/kg), Erythrosine for cocktail and candied cherries (200 mg/kg) and Litholrubine BK for edible coatings of cheeses (in specified quantities). For meat and meat products only a single dye may be used: Allura Red AC, Brilliant Blue FCF and Brown HT or a mixture of Allura Red AC and Brilliant Blue FCF. For the purpose of food colourings used in the home, the pigments and dyes listed in Tables 11.12 and 11.13 are available, except for annatto, canthaxanthin, Amaranth, Erythrosine, Brown FK, Litholrubine BK and aluminium pigment. The rules also specify foods that can be coloured only by certain dyes. For example, beer, vinegar, spirits of whiskey, brandy and rum-type alcoholic beverages, may be coloured with caramel (in a specified quantity), butter with carotene (10 mg/kg), margarines may be coloured with carotene and curcumin (in specified quantities) or annatto (10 mg/kg). Foods that cannot be coloured are also identified, mostly including unprocessed foods, such as milk, vegetable oils and animal fats, mineral and table water, egg contents, flour, bread, pasta products, sugar, meat, fish, wine, honey, vinegar, fruit and vegetable juices, tomato paste and sauces, coffee, cocoa and chocolate products, infant and child nutrition and others.

11.4.1.6 Health assessment

Food pigments and dyes should not represent any health risk if used up to the maximum amount allowed, as each substance authorised for use in the EU is subject to a rigorous scientific safety assessment. Some dyes can, however, cause health problems. Side effects are known for azo dyes, such as Tartrazine, Sunset Yellow

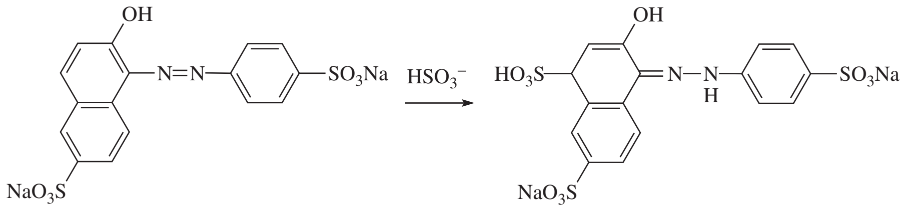


Figure 11.13 Reaction of bisulfite with Sunset Yellow FCF.

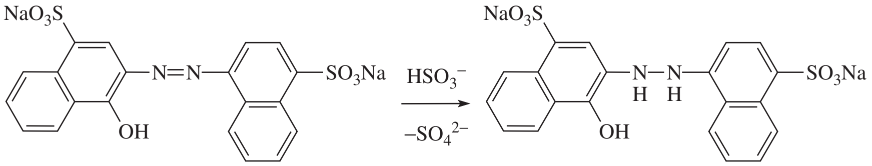


Figure 11.14 Reaction of bisulfite with Azorubine.

FCF, Azorubine and also other azo dyes, in people intolerant to salicylates (aspirin and some fruits). In combination with benzoates, these dyes are implicated in a large percentage of cases of hyperactivity in children, as well as carmine. Asthmatics may also experience symptoms following consumption of azo dyes, as they are histamine-liberating agents. Erythrosine may cause hyperactivity and increased photosensitivity in people with sensitivity to sunlight, and possible mutagenicity has also been reported. In high concentrations, Erythrosine may interfere with iodine metabolism, but these concentrations cannot be reached through the consumption of food. Patent Blue V and Indigotine can function as histamine-liberating agents, and may cause allergic reactions due to coupling of the colour to body proteins as well as Brilliant Blue FCF and Green S. Litholrubine BK is not absorbed and no side effects are known when it is used as a food additive.

A promising solution is the use of natural pigments extracted from tissue cultures. Other encouraging prospects are new dyes, such as high molecular weight pigments non-absorbable in the digestive tract, with chromophores fixed to the polymer so that they pass through the digestive tract in an unaltered state.

11.4.2 Bleaching agents

Bleaching agents include compounds that unwanted dyes:

- reduce
- oxidise to colourless or less intensely coloured products.

11.4.2.1 Reducing agents

Substances with reducing effects are sulfur dioxide and sulfites, which are also used as preservatives, inhibitors of enzymatic browning reactions and inhibitors of non-enzymatic browning reactions. The bleaching activity is mainly based on their ability to reduce the primary products of enzymatic browning reactions – quinones, whose subsequent reactions would otherwise lead to undesirable discoloration in dried fruits, vegetables, potatoes and other products. Furthermore, these compounds are used for the bleaching of hops, lecithin concentrates, mushrooms, nuts and fish products.

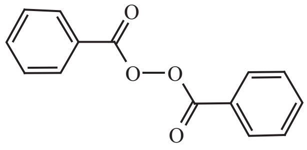
11.4.2.2 Oxidising agents

Substances with oxidising effects (not allowed under EU legislation) include:

- compounds with active oxygen
- compounds with active chlorine.

11.4.2.2.1 Compounds with active oxygen

Halides and peroxides have some importance as bleaching agents with active oxygen. The most widely used agent for bleaching flour is potassium bromate ($KBrO_3$), which has been used for about 90 years. Potassium bromate bleaches carotenoid pigments in flour and simultaneously oxidises gluten and glutathione, thus improving the baking properties of flour (see Section 5.14.6.1.6). It is reduced to bromide. The problem is its toxicity and carcinogenicity, therefore it was never been allowed as a flour improver in a number of countries, but is commonly used in many Latin American and East Asian countries. The suggested quantity of potassium bromate for improving bread flour is 50 mg/kg. Certain other compounds have also been used for the same purpose as potassium bromate. For example, inorganic compounds that are used include potassium and calcium iodates (IO_3^-), cupric sulfate, ammonium and potassium peroxodisulfates (also known as persulfates, $S_2O_8^{2-}$), hydrogen peroxide and ozone. Organic compounds used as bleaching agents and dough conditioners include dibenzoyl peroxide (**11-59**) and azodicarbonamide (azoformamide). Azodicarbonamide releasing nitrogen gas was previously used as a blowing agent in rubber and plastic products that were permitted in food packaging applications. In 2003, the European Food Safety Authority (EFSA) implicated foamed poly(vinyl chloride) cap liners as the source of potentially genotoxic semicarbazide (a group 3 substance not classifiable as to its carcinogenicity to humans according to International Agency for Research on Cancer, IARC). In certain countries, azodicarbonamide is still approved as a food additive up to a maximum of 45 mg/kg in flour, such as in the United States (at levels up to 45 mg/kg), Canada, and Asia, but is banned in Singapore, Australia and Europe. Azodicarbonamide is stable in dry flour, but it reacts in moist flour and yields, as the main reaction product biurea, which is relatively stable during baking, but partly decomposes to urazole, semicarbazide and unstable carbamic acid (Figure 11.15), which yields ethyl carbamate on reaction with the ethanol produced by yeast. Semicarbazide was not detected after room temperature or elevated temperature dough maturation, but only in bread. Commercial bread products show a wide range of semicarbazide concentrations (10–1200 μg/kg).



11-59, dibenzoylperoxide

11.4.2.2.2 Compounds with active chlorine

Gaseous chlorine, chlorine dioxide (ClO_2) and sodium and potassium hypochlorites (OCl^-) are used as bleaching agents and improvers of flour baking properties and also for chemical disinfection of water. The most frequently used agent is chlorine dioxide, in amounts of up to about 30 mg/kg flour in some countries.

The use of compounds with active chlorine is problematic from the hygienic and toxicological perspectives, because their reactions with food components may produce a number of potentially toxic chlorinated products. Chlorine dissolved in water reacts to form hypochlorous acid and hydrochloric acid:

$$Cl_2 + 2H_2O \rightleftharpoons HOCl + H_3O^+ + Cl^-$$

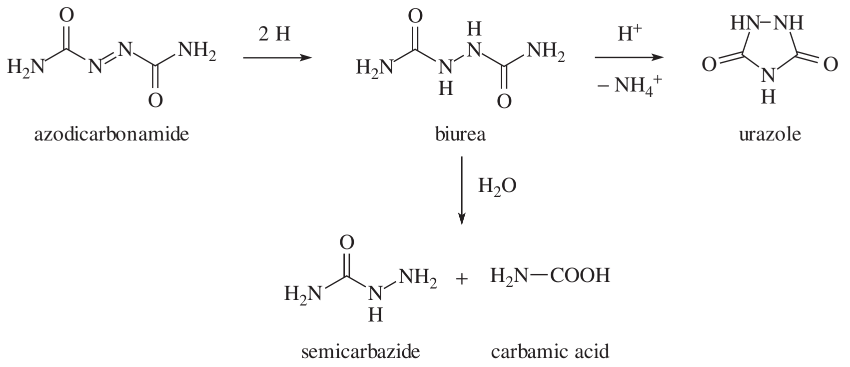


Figure 11.15 Decomposition of azodicarbonamide.

Under acidic conditions, the reaction equilibrium is shifted away from the formation of hypochlorous acid, which explains the formation of hypochlorite and chlorine and also the chlorinating action of chloramines, which are used as disinfectants and sanitising agents in the food industry. In acidic media hypochloric acid cation and chloride arise in the reaction with hydronium ions:

$$HOCl + H_3O^+ \rightleftharpoons H_2OCl^+ + H_2O \rightleftharpoons Cl^+ + 2H_2O$$

Hypochlorous acid dissociates with increasing pH (at pH 4–6 and higher):

$$Cl_2 + 2H_2O \rightleftharpoons HOCl + H_3O^+ + Cl^-$$

Hypochlorous acid and its anion also arise in alkaline media (pH 10.5–12.5):

$$Cl_2 + HO^- \rightleftharpoons HOCl + Cl^-$$
$$HOCl + HO^- \rightleftharpoons ClO^- + H_2O$$

In addition to these reactions, there irreversible reactions also proceed, in which oxygen is formed as the reaction product, which explains the oxidative properties of chlorine, hypochlorites and chloramines:

$$2Cl_2 + 2HO^- + 2H_2O \rightarrow 4Cl^- + 2H_3O^+ + O_2$$
$$\text{or } 2ClO^- \rightarrow 2Cl^- + O_2$$
$$2Cl^+ + 4HO^- \rightarrow 2Cl^- + 2H_2O + O_2$$

In the reaction of chlorine dioxide with water, hypochlorous, hydrochloric and chloric acids are formed temporarily, and in alkaline solutions chlorites (ClO_2^-), chlorates (ClO_3^-) and other products arise. The cation H_2OCl^+ formed in aqueous solutions of chlorine, chlorine dioxide and hypochlorites may react with alkenes and other unsaturated compounds. The electrophilic addition of HOCl to alkenes is an established reaction mechanism for α, β-chloroalcohol (chlorohydrin) and α, β-dichloro derivative formation (Figure 11.16). The reaction yielding chlorohydrins follows the Markovnikov rule with the hydroxyl group adding to the more substituted carbon. Oxidation of chlorohydrins by hypochlorites

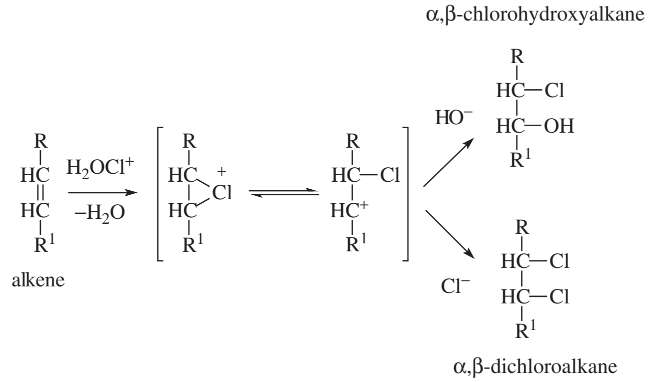


Figure 11.16 Reactions of hypochlorites with olefins.

in acidic media may yield α-oxochloroalkanes, while epoxides arise in neutral (or slightly alkaline) media, by elimination of hydrogen chloride, and are hydrolysed by acids and bases to the corresponding α, β-dihydroxyalkanes.

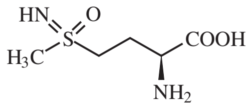
The reaction of chlorine in flour with unsaturated fatty acids, which are also alkenes, yields a number of fatty acid chlorinated derivatives (dichloroacids and chlorohydroxyacids). Chlorohydroxyacids are formed preferentially with chlorine dioxide. For example, oleic acid produces 9,10-dichlorostearic, 9-chloro-10-hydroxystearic and 10-chloro-9-hydroxystearic acids. From linoleic acid the corresponding disubstituted derivatives of oleic acid or tetrasubstituted derivatives of stearic acid are formed. Their content in flour, depending on the amount of chlorine used for bleaching, is given in Table 11.14. Chlorinated fatty acids are also formed in the fat of chicken carcasses that are cooled in chlorinated water after slaughter.

The use of nitrogen trichloride, also known as trichloroamine (NCl_3), to bleach flour was found to evoke hysteria in experimental animals (dogs), due to the presence of methionine sulfoximine (**11-60**). Methionine sulfoximine, a methionine antagonist, arises in the reaction of methionine with nitrogen trichloride.

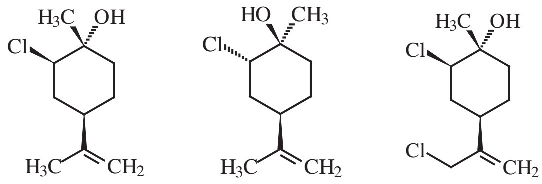
Unsaturated terpenes also react with electrophilic reagents, such as hypochlorous acid (HOCl). Reactions of HOCl with a variety of terpenes, including limonene monoxide, α-pinene and α-terpineol, have been reported. For example, limonene, the

Table 11.14 Chlorinated fatty acids in flour bleached with chlorine.

Chlorine in flour	Chlorinated fatty acids (mg/kg of flour)					
(mg/kg)	$C_{18:1}$(Cl, Cl)	$C_{18:0}$(Cl, Cl)	$C_{18:0}$(Cl, Cl, Cl, Cl)	$C_{18:1}$(OH, Cl)	$C_{18:0}$(OH, Cl)	$C_{18:0}$(OH, Cl, Cl, Cl)
0	0	0	0	2	< 1	0
100	7	0	0	37	6	0
500	130	12	2	170	28	22
1000	330	75	39	160	65	130
2000	100	160	210	26	71	540
3000	50	160	240	41	160	1010



11-60, methionine sulfoximine



(1*R*,2*R*,4*R*)-2-chloro-8-*p*-menthen-1-ol (1*S*,2*S*,4*R*)-2-chloro-8-*p*-menthen-1-ol (1*R*,2*R*,4*R*)-2,9-dichloro-8-*p*-menthen-1-ol

11-61, reaction products of limonene with hypochlorous acid

major component of orange essential oil, reacts with hypochlorous acid found in chlorinated water to form the diequatorially substituted (1*R*, 2*R*, 4*R*)-2-chloro-8-*p*-menthen-1-ol, the diaxial (*E*)-stereoisomer, (1*S*, 2*S*, 4*R*)-2-chloro-8-*p*-menthen-1-ol as the major chlorohydrin and the dichlorohydrin, (1*R*, 2*R*, 4*R*)-2,9-dichloro-8-*p*-menthen-1-ol (**11-61**).

One of the major problems associated with the disinfection of water supplies by chlorination is that the chlorinated water may produce the so-called chlorophenolic taste produced by a reaction between the added chlorine and phenol and some of its homologs that are present in trace amounts. The chlorination of phenol proceeds by the stepwise substitution of the 2-, 4- and 6-positions of the aromatic ring. Initially, phenol is chlorinated to form either 2- or 4-chlorophenol. Then 2-chlorophenol is chlorinated to form either 2,4- or 2,6-dichlorophenol, while 4-chlorophenol forms 2,4-dichlorophenol. Both 2,4- and 2,6-dichlorophenol are chlorinated to form 2,4,6-trichlorophenol, which reacts with aqueous chlorine to form a mixture of non-phenolic oxidation products. 2,4,6-Trichloroanisole was the first compound identified as the source of taints in wines, perceived as musty or mouldy aromas. 2,4,6-Trichloroanisole in wine originates from chlorination of lignin-related substances during bleaching of the cork with chlorine, the product(s) subsequently being leached into the wine during storage. This taint is known as corkiness and is widely attributed to the interaction of bacteria and fungi with constituents of the cork that methylate 2,4,6-trichlorophenol. 2,4,6-Trichloroanisole can be similarly formed during microbial contamination of packaging materials and in soil contaminated with phenols. 2,4,6-Trichlorophenol is also an industrial agent used to decontaminate wooden objects, including the floors, beams and barrels in wine cellars. In addition, 2,4,6-trichloroanisole is produced together with 2,4,6-trichlorophenol by degradation of some pesticides (such as fungicide pentachlorophenol). Pentachlorophenol and the lower chlorinated phenols, tetra- and trichlorophenols, have been used increasingly as fungicides, herbicides, insecticides and precursors in the synthesis of other pesticides since the early 1930s (see Section 12.6.2.1.1).

11.5 Substances modifying texture

Substances modifying and regulating food texture and other physical properties of foods are, in terms of quantity, the main additive substances used. The most important groups are:

- thickeners and gelling agents
- emulsifiers.

Many of these substances can be simultaneously classified into several categories of food additives, because they have different properties and are used for various purposes.

11.5.1 Thickeners and gelling agents

The reason for the use of thickeners and gelling agents is to create and maintain a desirable texture to food. Thickeners are substances that increase the viscosity of food, and gelling agents

produce gels. These additives include natural plant polysaccharides, such as starch (not considered a food additive), cellulose (E460) and pectins (E440), seaweed polysaccharides, such as gum arabic (E414), agar (E406) and carrageenan (E407), extracellular bacterial polysaccharides, such as gellan (E418) and xanthan gums (E415) and modified polysaccharides, such as modified starches (E1404, E1410, E412-E1414, E1420, E1422, E1440-1442) and modified celluloses (E461–E469). Their structure, occurrence, properties and applications are given in the Chapter 4 dealing with polysaccharides. Gels and other food dispersion systems are described in Section 7.8.3.2.2.

11.5.1.1 Legislation

Thickeners and stabilisers of dispersions and emulsions, which can be used to the maximum amount allowed are, for example, karaya gum (E416), konjak gum (E425), sucrose acetate isobutyrate (E444) and glycerol esters of wood rosins (E445). For example, karaya gum (E416) is allowed at levels of 5000 mg/kg for chewing gums, confectionery fillings, toppings for pastries and biscuits, at 6000 mg/kg for desserts and up to 10 000 mg/kg for cold emulsified sauces and egg liqueurs.

11.5.1.2 Health assessment

Some thickeners and gelling agents are considered as food (e.g. starches) and for many others ADI values are not specified, for example for carrageenan (E407), locust bean gum (E410), guar gum (E412), tragacanth (E413), powdered and microcrystalline cellulose (E460), gum arabic (E414) and many others. ADI values are set for karaya gum (20 mg/kg), tara gum (E417, 12.5 mg/kg), methyl, ethyl, hydroxymethyl, hydroxypropyl and sodium carboxymethyl cellulose (25 mg/kg) and dextrins (E1400, 70 mg/kg). The role of thickeners and gelling agents in nutrition and other information are provided elsewhere (see Section 7.8.3.2.2). No side effects of natural polysaccharides, modified starches and celluloses in the concentrations used are known, although high concentrations may bring about flatulence and bloating, due to fermentation by intestinal microflora (in the same way as all indigestible polysaccharides) and high concentrations of cellulose (E460), and modified celluloses can cause intestinal problems, such as bloating, constipation and diarrhoea. Tara gum (E417) and gellan gum (E418) may have laxative properties. Short-chain carrageenans may cause intestinal leakage and are not permitted for use in foods. High concentrations of alginic acid and alginates (E400) could lead to impairment of iron uptake, as iron is efficiently bound by these polysaccharides.

11.5.2 Emulsifiers

Emulsifiers (also known as emulgents) are surfactants enabling the formation of emulsions (especially dispersions of fat in various products). In addition to their ability to form an emulsion, emulsifiers have the ability to interact with other food ingredients. The emulsifier may be an aerating agent, starch complexing agent and/or crystallisation inhibitor. In flours they act as conditioners that soften the crust of pastry, and in confectionery they act as modifiers of crystallisation of fats and have other beneficial properties. Formation of emulsions, their properties and the factors affecting their formation are described in Section 7.8.3.3.

11.5.2.1 Classification

Food emulsifiers are classified according to several criteria. Any emulsifier consists of a hydrophilic head derived from a variety of polar compounds (e.g. glycols and sugar alcohols) and a hydrophobic tail, which is a residue of a fatty acid. The hydrophilic head is directed to the aqueous phase and the hydrophobic tail to the oil. According to the structure of the polar part of the molecule (hydrophilic head), the following groups of emulsifiers are recognised:

- esters of glycols (e.g. esters of propane-1,2-diol)
- glycerol esters and their derivatives (e.g. partial esters of glycerol)
- esters of sorbitans (esters of glucitol dehydration products)
- sucrose esters (partial esters)
- esters of hydroxycarboxylic acids (such as lactic and tartaric acids)
- lecithin and its derivatives.

According to the origin, emulsifiers are identified as:

- natural (such as lecithin and partial esters of glycerol)
- synthetic (other emulsifiers).

According to the properties of hydrophilic and lipophilic moieties (expressed by the so-called HLB value) emulsifiers are recognised as:

- hydrophilic
- lipophilic.

According to their ability to form or not to form ions, emulsifiers are classified into:

- ionogenic also known as ionic (the hydrophilic moiety can be an anion, a cation or may have an amphoteric character)
- non-ionogenic also known as non-ionic (the hydrophilic part of the molecule is not ionised).

Salts of fatty acids are ionogenic emulsifiers and lecithins have an amphoteric character. The most common food emulsifiers (e.g. fatty acid esters of glycerol, sorbitans, sucrose and hydroxycarboxylic acids) are non-ionogenic emulsifiers.

Table 11.15 HLB values and E-numbers of selected emulsifiers.

Substance	HLB	Substance	HLB
Oleic acid	1.0	Polyoxyethylene sorbitan tristearate (Tween 65, E436)	10.5
Sorbitan tristearate (Span 65, E492)	2.1	Polyoxyethylene sorbitan monostearate (Tween 60, E435)	14.9
Monostearoylglycerol (mixture of isomers)	3.4	Polyoxyethylene sorbitan monooleate (Tween 80, E433)	15.0
Sorbitan monooleate (Span 80, E494)	4.3	Polyoxyethylene sorbitan monopalmitate (Tween 40, E434)	15.6
Sorbitan monostearate (Span 60, E491)	4,7	Polyoxyethylene sorbitan monolaurate (Tween 20, E432)	16.7
Sorbitan monopalmitate (Span 40, E495)	6.7	Sodium oleate	18.0
Sorbitan monolaurate (Span 20, E493)	8.3	Potassium oleate	20.0

The applicability of food non-ionogenic emulsifiers greatly depends on their HLB value (Hydrophilic Lipophilic Balance value), which is a measure of the degree to which the emulsifier is hydrophilic or lipophilic. Substances with a low HLB value tend to dissolve in oil (o), but substances with a high HLB value dissolve better in water (w). Numerical HLB values range from 0.0 to 20.0, with a value of 0 corresponding to lipophilic substances and value of 20 to hydrophilic substances – see Table 11.15).

11.5.2.1.1 Lecithin and its derivatives

The term lecithin is used for (3-*sn*-phosphatidyl)choline (1,2-diacyl-*sn*-glycero-3-phosphocholine, or phosphatidylcholine for short) and the mixture of natural phospholipids used as emulsifiers (see Section 3.5.1). Crude food grade lecithins (E322) are mainly obtained from the processing (degumming, also known as hydration) of vegetable oils (soybean, maize, or safflower oils). Almost all of the commercially available lecithin is derived from soybean oil, which contains 1–3% of lecithin. Less important sources include other vegetable oils and eggs. The main component of lecithin is phosphatidylcholine, where the hydrophilic part of the molecule $X = (CH_2)_2N^+(CH_3)_3$. Phosphatidylcholine is accompanied by phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol (see Section 3.5.1). Commercial lecithin may contain up to 35% triacylglycerols.

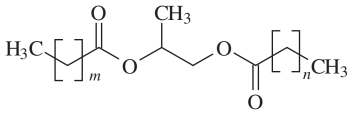
Commercial lecithin is an important product, because of its dietary significance and multifaceted industrial applications. Lecithin products fall into three categories: natural lecithin (such as unbleached and bleached lecithin), refined lecithin and chemically modified lecithin. Bleaching of dark brown crude lecithin is carried out using hydrogen peroxide or benzoyl peroxide to obtain a product of lighter colour, but may result in peroxide oxidation of unsaturated fatty acids. Chemically modified lecithin is more hydrophilic. For example, hydroxylated lecithin is prepared by treating lecithin with hydrogen peroxide and acetic or lactic acid. Two of the earliest applications of lecithin include lowering the viscosity in chocolate and confectionery products, and emulsification/anti-spatter properties in margarine. In addition, its other uses are in the production of bakery goods, pasta products, textiles, insecticides and paints.

11.5.2.1.2 Fatty acid salts

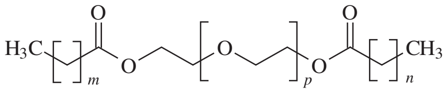
Salts of fatty acids (sodium, potassium and calcium salts, E470a, or magnesium salts, E470b) are produced mainly from vegetable oils, but can also be produced from animal fats. The acids are a mixture of oleic, palmitic, stearic and myristic acids. Salts of fatty acids are used as anti-caking agents in powdered foods to prevent clumping and as emulsifiers.

11.5.2.1.3 Esters of glycols

Fatty acid esters of propane-1,2-diol (E477), also known as propylene glycol (**11-62**), and fatty acid esters of polyethylene glycol 8000 (**11-63**, E1521, the HLB value depends on the degree of ethoxylation) are obtained by direct esterification or by enzymatic reactions. These substances are used for oil-in-water emulsions (o/w emulsions for short).



11-62, propyleneglycol diesters



11-63, polyethyleneglycol diesters

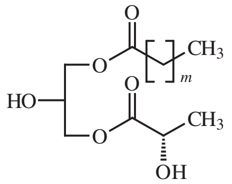
11.5.2.1.4 Esters of glycerol and their derivatives

Monoacylglycerols and diacylglycerols

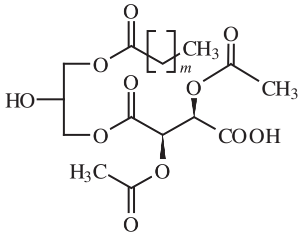
Partial esters of glycerol, mono- and diacylglycerols (E471), are obtained by glycerolysis of fats (usually hydrogenated oils) or by direct esterification of glycerol. The predominant components of monoacylglycerols are 1-acyl-*sn*-glycerols and diacylglycerols are dominated by 1,3-diacyl-*sn*-glycerols. Monoacylglycerols obtained by molecular distillation (containing about 90% of monoacylglycerols) are suitable for most purposes.

Esters of monoacylglycerols and diacylglycerols

The emulsifying efficiency of partial glycerol esters is higher when these esters are esterified with carboxylic acids, such as acetic (E472a), lactic (E472b), citric (E472c), fumaric, succinic, tartaric (E472d), monoacetyl and diacetyl tartaric acids (E472e) or mixtures thereof, such as mixed acetic and tartaric acid esters of mono- and diacylglycerols (E472f). For example, the reaction of 1-acyl-*sn*-glycerols with lactic acid yields a mixture of products containing 1-acyl-3-lactate (**11-64**), its isomer 1-acyl-2-lactate and fully esterified product 1-acyl-2,3-dilactate. Corresponding products are produced with diacetyl tartaric acid (**11-65**). Analogously to these derivatives containing one fatty acid in the molecule, produced for special purposes are esters of glycerol with two fatty acids and one hydroxycarboxylic acid (esters of diacylglycerols with hydroxycarboxylic acids).



11-64, (*S*)-1-acyl-3-lactoyl-*sn*-glycerol

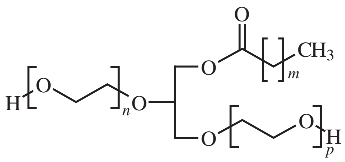


11-65, (2*R*,3*R*)-1-acyl-3-(2,3-diacetyltartaroyl)-*sn*-glycerol

Esters of mono- and diacylglycerols with acetic acid (E472a) are used as substances preventing crystallisation of fats, esters of succinic acid have found use as flour conditioners (enhancers), esters of citric acid as emulsifiers, solvents for antioxidants and fat substitutes in some foods. They also enhance the baking properties of flour.

Ethers of monoacylglycerols and diacylglycerols

Another way of modifying the properties of partial esters of glycerol is their reaction with ethylene oxide in alkaline media (ethoxylation), which yields products with different lengths of side chains (that contain up to 40 residues of ethylene oxide), sometimes branched and therefore of different polarities. The structure of 1-monoacyl-*sn*-glycerol ethers is represented by the formula **11-66**. Instead of ethylene oxide propylene oxide may similarly be used, which gives rise to analogous products. Mixed copolymers may also be obtained. Ethoxylated monoacylglycerols, for example, have good dough strengthening characteristics but very little crumb softening. Dosage of these viscous liquids is rather critical as excess amounts cause excessive expansion which can lead to collapse of the bread when in the oven. Ethoxylated monoacylglycerols are usually combined with monoacylglycerols, but they are not approved for use in the EU.



11-66, polyethyleneglycol ether of 1-monoacyl-*sn*-glycerol

Esters of polyglycerols

Fatty acids can also be esterified by polyglycerols (E475), which are formed from glycerol in alkaline media via 2,3-epoxypropan-1-ol (glycidol, Figure 11.17). Glycidol can isomerise to 3-hydroxypropanal or 1-hydroxypropanone, which give various coloured products in the Maillard reaction. Glycidol additionally condenses with another molecule of glycerol to 1,1-diglycerol (4-oxa-heptane-1,2,6,7-tetrol), to a lesser extent to 1,2-diglycerol (3-oxa-2-hydroxymethylhexan-1,5,6-triol) or, via epoxide, to triacylglycerols (such as 4,8-dioxaundecan-1,2,6,10,11-pentol) and higher oligomers. The dominant products under alkaline conditions are diglycerols, but also common are products that contain 3–6 or more glycerol molecules. In addition to linear products, to a lesser extent (reactivity of secondary hydroxyl groups are lower) branched products are formed. In acidic media various cyclic products are produced, of which the main product is 2,5-bis(hydroxymethyl)-1,4-dioxane formed as a product of diglycerol dehydration. The same products result from glycerol in acid protein hydrolysates.

Phosphatidic acids

Monoacylglycerols and diacylglycerols can also be esterified using phosphorus pentoxide. For example, esterification of 1,2-diacyl-*sn*-glycerol yields phosphatidic (1,2-diacyl-*sn*-glycerol 3-phosphate) and the corresponding bisphosphatidic acid and their positional isomers. Both acids are natural-identical products, which serve as substitutes for lecithin. Ammonium phosphatides (E442) are approved in the EU as emulsifiers.

11.5.2.1.5 Derivatives of sorbitans

Sorbitan esters

An important group of non-ionogenic emulsifiers are esters of sorbitol (correctly D-glucitol) with higher fatty acids. During esterification, sorbitol is simultaneously dehydrated, creating a mixture of free anhydro derivatives called sorbitans, their monoesters, diesters and triesters. The most important products are esters derived from 1,4-sorbitan, 1,5-sorbitan and 2,5-sorbitan (**11-67** to **11-69**). The reaction can be continued to yield a dianhydride ester, which is called isosorbide (**11-70**). The resulting mixtures of

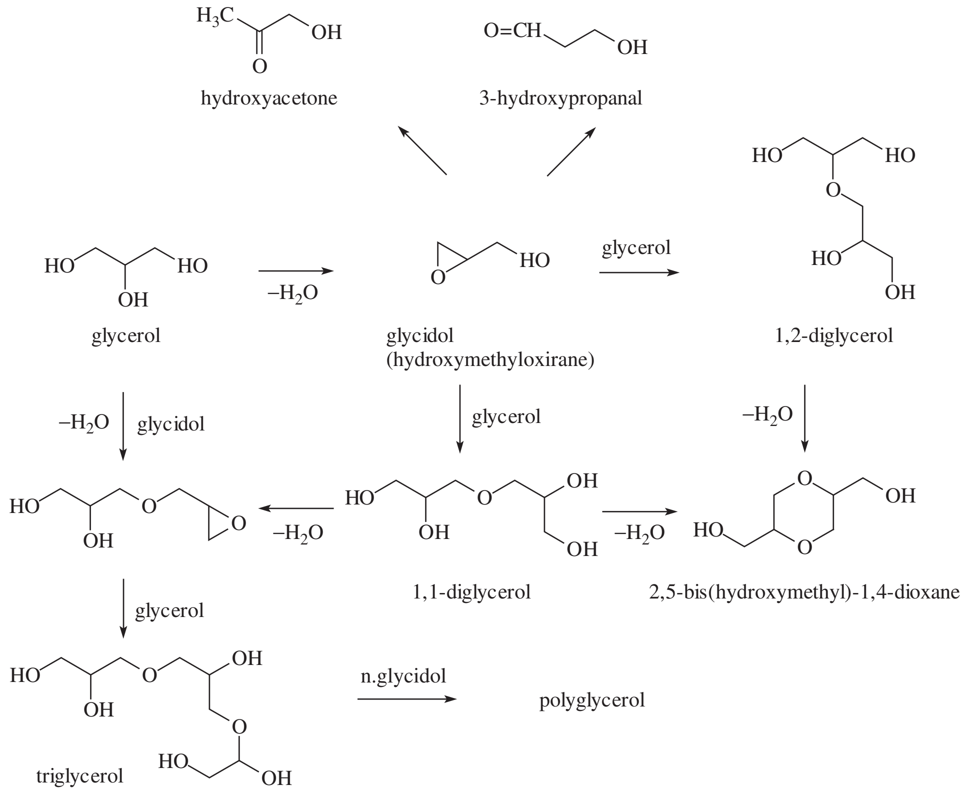
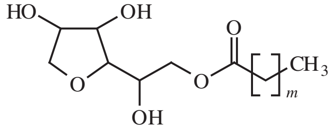
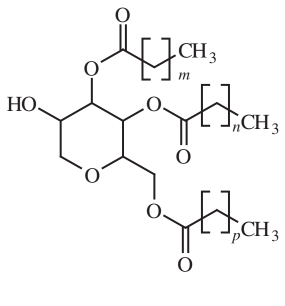


Figure 11.17 Reactions of glycerol with glycidol.

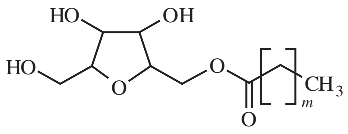
various mono-, di- and triesters of optically active anhydrides (e.g. the corresponding 1,4-sorbitan structure is represented by the formula **11-71** and the isosorbide structure by the formula **11-72**) are known as **Spans**. Their trade names, E-numbers and HLB values are listed in Table 11.15. Spans act as lipophilic emulsifiers with properties similar to those of monoacylglycerols (sorbitan monoesters) and diacylglycerols (sorbitan triesters), but with higher emulsifying ability.



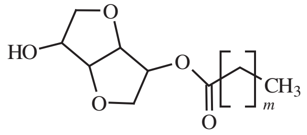
11-67, 1,4-sorbitan ester



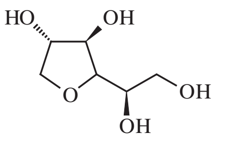
11-68, 1,5-sorbitan triester



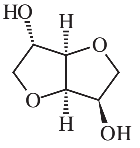
11-69, 2,5-sorbitan ester



11-70, isosorbide ester



11-71, 1,4-sorbitan

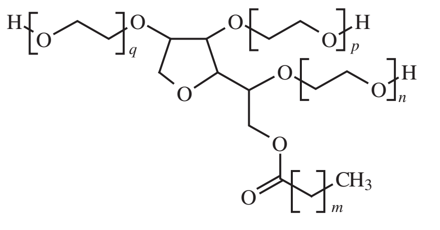


11-72, isosorbide

Acylsorbitan ethers

Partial esters of sorbitans with fatty acids can be modified by reaction with ethylene oxide (under pressure, at elevated temperatures and in the presence of sodium ethoxide or other catalysts), which yields a mixture of products substituted to varying degrees

in free hydroxyl groups, the side chains of which may be branched. These polar emulsifiers are also known as polysorbates or **Tweens** (**11-73**). They are very effective in slowing down the aging of pastry products. Their trade names, E-numbers and HLB values are listed in Table 11.15.

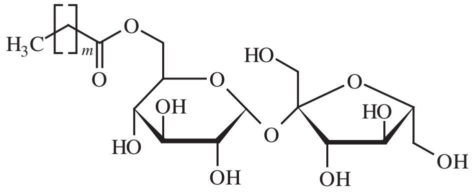


11-73, Tween derived from 1,4-sorbitan

11.5.2.1.6 Sucrose derivatives

Esters

A special group of emulsifiers are sucrose esters of higher fatty acids (E473), which are synthesised from fatty acid methyl esters re-esterified by sucrose (in dimethylformamide or dimethyl sulfoxide under catalysis of alkaline reagents). The most common compounds are strongly polar, water-soluble monoesters (**11-74**) with HLB values > 16, which are used as stabilisers of emulsions, or diesters, which have a wide range of HLB values ranging from 7 to 13 and are used to stabilise the emulsions of the type o/w. Adjusting the reaction conditions can also provide triesters (HLB < 1) and tetraesters. Like sucrose, sucrose esters are easily decomposed on heating to give coloured products.



11-74, sucrose-6-ester

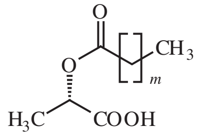
Sucrose esters containing more than five fatty acid residues do not have emulsifying ability, but can be used as low-energy fat substitutes, because they are not absorbed in the digestive tract. Such a product is Olestra, also known by its brand name Olean, which was approved by the US Food and Drug Administration (FDA) as a food additive in 1996. In the late 1990s, Olestra lost its popularity due to the discovery of side effects (including inhibition of absorption of some fat-soluble vitamins) and is not approved for sale in many countries.

Sugarglycerides

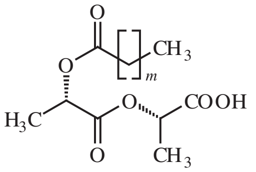
Esterification of glycerol esters by sucrose yields the so-called sugarglycerides (E474), which represent a mixture of various sucrose esters and glycerol esters. Their polarity roughly corresponds to the polarity of monoacylglycerols.

11.5.2.1.7 Hydroxycarboxylic acid esters

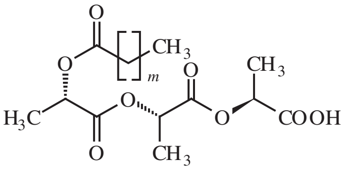
Fatty acids may also be directly esterified by hydroxycarboxylic acids, the most common of which are lactic acid and (2*S*,3*S*)-tartaric (D-tartaric) acid. Lactic acid gives rise to esters called lactylates, at first a monoester (**11-75**), which reacts with another molecule of lactic acid yielding the ester of a dimeric acid (**11-76**), which may arise also by reaction of a fatty acid with lactides. Other reactions can produce emulsifiers in which one molecule of a fatty acid accounts for a greater number of molecules of lactic acid (**11-77**). Stearoyl tartrate, also known as stearoylpalmitoyl tartrate (E483), is approved in the EU as an emulsifier. The main components of this product are distearoyl tartrate, dipalmitoyl tartrate and stearoylpalmitoyl tartrate.



11-75, L-lactic acid ester



11-76, ester of lactic acid dimer



11-77, ester of lactic acid trimer

Esters of lactic acid (lactylates) can be converted into salts and thereby become highly effective polar, anionic emulsifiers. Sodium (E481) and calcium (E482) stearoyl 2-lactylates are approved in the EU as emulsifiers and flour improvers.

11.5.2.1.8 Other emulsifiers

Fatty acids can be esterified by a number of sugar alcohols (such as D-mannitol, maltitol and lactitol) or sugars (D-glucose, D-fructose, maltose and lactose).

11.5.2.2 Properties

For application in food systems and preparation of w/o emulsions, emulsifiers with an HLB value of 3–6 are used, while for the preparation of o/w emulsions substances with an HLB value of 15–18 are used. Emulsifiers with an HLB value of 7–9 are commonly used as moisturisers (emollients). Approximate HLB values of certain emulsifiers and other surfactants are given in Table 11.15. For example, an emulsifier of HLB value 17 is needed for the preparation of an o/w emulsion of oleic acid, an emulsifier of HLB value 9 for beeswax emulsion, an emulsifier of HLB value 6 for rapeseed oil and cocoa butter emulsions and an emulsifier of HLB value 5 for pork lard emulsion. In practice, mixtures of two compatible

emulsifiers in calculated proportions (one with low HLB value and the other with high HLB value) are preferred.

11.5.2.3 Legislation

Emulsifiers which may be used in the necessary quantities are emulsifiers E322, E471 and E472a–f. Polysorbates (E432–E436), sugar esters (E473, E474), stearoyl lactates (E481, E482) and emulsifiers E475, E476, E477, E479b, E483 can only be used up to the maximum amount permitted for certain foods. For example, sugar esters E474 are allowed for fine pastry, biscuits and confectionery products in quantities up to 10 000 mg/kg, for ice creams in quantities up to 5000 mg/kg and for soups and broths in quantities up to 2000 mg/kg. The maximum amount allowed varies according to the type of emulsifier and type of food. For example, for polyoxyethylene sorbitans this amount ranges from 1000 (dietary foods for weight control) to 10 000 mg/kg (emulsified fats for bakery purposes).

11.5.2.4 Health assessment

There is no evidence in the available information on lecithin and lecithin bleached with hydrogen peroxide that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future. For fatty acid salts, monoacylglycerols and diacylglycerols, esters of mono- and diacylglycerols with acetic, lactic and citric acids, no acute or chronic effects have been observed, and ADI values are not specified.

Certain toxic effects may be observed at higher doses of other emulsifiers, but side effects are not known in the concentrations used. ADI values have been set for the remaining emulsifiers: 25 mg/kg body weight for propane-1,2-diol esters and polyglycerols esters of fatty acids (high concentrations of propyleneglycol can cause eczema in sensitive persons, but not normally from the use in foods), none for esters of mono- and diacylglycerols with carboxylic acids, except 30 mg/kg body weight for tartaric acid esters (E472d-E472f), 30 mg/kg body weight for ammonium phosphatides, 25 mg/kg body weight for sorbitan esters (Spans) and for the entire group of polyoxyethylene sorbitans (Tweens) in the E432–E436 range (people intolerant of propylene glycol should also avoid this group of emulsifiers), 16 mg/kg body weight for sucrose esters and sucroglycerides and 20 mg/kg body weight for stearoyl tartrate and sodium and calcium stearoyl lactates.

11.6 Substances increasing biological value

Important nutritional factors include vitamins, minerals, amino acids, some fatty acids, fibre and other substances with important biological effects. Some of these substances may be used as food supplements that increase the nutritional value of the food or show other beneficial effects, can be also used as food additives and may have a function as pigments (riboflavin) or antioxidants (such as ascorbic acid). The use of substances enhancing the biological value of food closely monitors the development of knowledge in nutrition and is focused to intake of certain essential exogenous substances that may prevent various, previously endemic or just regional, diseases. Two basic reasons exist for the use of dietary supplements:

- they preserve the nutritional quality of food consumed at levels consistent with modern knowledge (such as adding vitamin D to margarines)
- they correct the deficiency of some nutritionally valuable substances in the diet (such as iodination of table salt).

11.6.1 Legislation

The Recommended Daily Allowances (RDAs) for vitamins and mineral elements set in the EU and U.S. are given in Sections 5.0 and 7.0, respectively. According to the European Regulation (EC) No 1925/2006, the enrichment of foods with vitamins and mineral elements is only allowed with approved (listed) vitamins, vitamin formulations, minerals and mineral substances and to a maximum per cent proportion of the reference daily intake. For the majority of vitamins and minerals, Directive 90/496/EEC on nutrition labelling of foodstuffs applies and defines a significant amount as 15% of the RDA.

11.6.2 Health assessment

Medical evaluation and other aspects of dietary supplements are listed in the chapters dealing with main nutrients (amino acids, essential fatty acids), vitamins and minerals.

11.7 Other food additives

In food production many other additives are used that have different properties and effects. Most often, this category of substances is classified into:

- firming agents
- processing aids
- synergists and potentiators
- propellants
- solvents.

Some technologies have identified other groups of additives. For example, for tobacco products combustion modifiers (activated carbon and ammonium chloride), substances for direct printing on cigarette paper, additives for chewing and snuff tobacco and others are used.

11.7.1 Firming agents

Firming agents are those that restore or maintain the texture of food. Generally they are soluble compounds that penetrate well into the material and do not have their own aroma and colour. These substances are mainly used in canned fruits and vegetables, jams and other products of plant origin, but also for animal products (such as cheeses). Typical firming agents are calcium and magnesium salts, such as calcium carbonate (E170), calcium hydrogen sulfite (E227), calcium citrates (E333), calcium phosphates (E341), calcium sulfate (E516), calcium chloride (E509), calcium gluconate (E578), magnesium chloride (E511), magnesium sulfate (E518) and magnesium gluconate (E580). For example, the effect of calcium chloride is based on the interaction of calcium ions with pectin, but it also binds metals and acts as an acidity regulator. The effect of sucrose is based upon its interaction with pectin substances, cellulose and hemicelluloses of plant cell walls by means of hydrogen bonds.

11.7.2 Processing aids

This category of additives includes:

- aroma carriers
- filling agents
- adhesives
- finishing substances
- humectants and plasticisers.

The use of solid aroma carriers facilitates the application of delicate or water-insoluble additives (particularly essential oils and various aroma compositions) in products. Aroma carriers also enhance the retention of aroma, since foods can be flavoured after heat or other treatments, which minimises the loss of aromatic compounds. Examples of aromatic carriers are starch, dextrins (E1400), cellulose (E460), silica (silicon dioxide, E551), and in particular β-cyclodextrin (E459) in quantities up to 1000 mg/kg. Aromatic substances are also applied after dissolution in appropriate solvents.

Filling agents increase the volume or weight of the food and generally do not significantly affect its energy yield. Fillers do not have own flavour and do not change colour of products. Some oligosaccharides and polysaccharides have found use in the manufacture of confectionery, chewing gum, vitamin preparations and cereal mixtures, and especially in various dietary and low-energy products. Fillers can also include some flour conditioners (others than emulsifiers), which increase the volume of bakery products, such as calcium stearoyl 2-lactylate (E482).

Adhesives bind food particles (e.g. reconstituted, poultry and fish meat and the so-called soy meat). They have also found use in extruded foods, production of chewing gum, sweets and tablets. The most commonly used additives are starch, dextrins (E1400), various plant gums, as well as oils and some salts (such as phosphates, which increase the solubility of proteins that denature during subsequent heat treatments and strengthen the material). Various adhesive agents are also used for food packaging.

Films on the food surface are often protection against oxidation by oxygen and slow down other reactions occurring in foods, prevent evaporation of water or (on the contrary) wetting and facilitate dissolution of products. Glossy coatings provide attractive appearances to the food. In some cases these films and coatings are a barrier from the invasion of microorganisms. Edible coatings or coatings which are easily removable are not considered as coatings. Some additives are used as glazing agents for fresh fruits (such as carnauba wax, E903), chocolates (such as a synthetic mixture of hydrocarbons – microcrystalline wax, E905) and eggs (to prevent access of air through pores of the shell – mineral oil, E905a). In the production of milk substitutes (coffee creamers) sodium caseinate for fat encapsulation is used.

Plasticisers are substances that affect the mechanical properties of food. Monoacylglycerols, oils, waxes and resins, and in particular, various wetting agents (humectants) are used as plasticisers. Humectants retain water in the food, prevent foods from drying out, reduce volatilisation of odorous substances or promote the dissolution of some substances in aqueous media. Polyols are mainly used as humectants. For example, glycerol (E422) is used in grated coconut, propane-1,2-diol (E477), glycerol, butane-1,3-diol, triethylene glycol ($HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$) and triacetin (triacetyl-*sn*-glycerol, E1518) in tobacco products, monoacylglycerols in caramel and margarine and waxes in chewing gums. Related groups are substances that increase the water-binding capacity in foods containing proteins, such as phosphates in dairy and meat products. Softening agents are used for printing inks and varnishes for packaging materials (such as triacetin in paper for cigarette filters).

11.7.3 Auxiliary agents

Auxiliary food additives include:

- anti-caking agents
- clarifying agents
- haze forming agents
- foaming agents
- dispersion stabilisers
- anti-foaming agents
- lubricants and release agents
- sequestrants
- synergists and potentiators
- packaging gases
- catalysts.

Anti-caking agents form coatings on the surface of the food particles and reduce their tendency to mutual adhesion. Potassium (E340) and magnesium phosphates (E343), silicon dioxide (silica, E551), various silicates (E551-E556, E559) and other substances may be used as anti-caking agents.

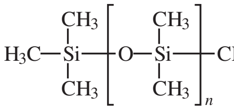
Clarifying agents stabilise beverages by removing hazes (e.g. in beer, wine and fruit juices). Proteins (such as gelatine), polyphenols (such as tannin), polyvinylpolypyrrolidone (E1202), phytic acid and bentonite (E558) can act as clarifying agents. Some enzymes that hydrolyse polysaccharides (such as pectins in fruit juices) are also clarifying agents.

To induce a turbid appearance to non-alcoholic drinks and beverages, especially to citrus fruit beverages, ice creams and other products, vegetable gums or, in the past, brominated vegetable oils (E443) are used. These are no longer approved in the EU. For beverages derived from fruits other than citrus fruits, the pulp and peels of citrus fruits is used.

Foaming agents are surfactants that allow creation of dispersions of gaseous substances in liquid or solid food. Foaming gases are carbon monoxide and carbon dioxide (E290) and in some countries natural saponins.

Dispersion stabilisers help to maintain desirable physical properties of emulsions and other disperse systems. Various polysaccharides, such as gum arabic (E414), are used as dispersion stabilisers.

Anti-foaming agents are food additives that prevent the formation of foam or reduce foaming, for example fatty acid esters of polyoxyethylene sorbitans (E432–E436) and silicone oils (dimethylpolysiloxane, E900, **11-78**).



11-78, dimethylpolysiloxane

Lubricants and releasing agents are applied on the surface of products or on the surface of production equipment. The purpose is to reduce the mutual attractive forces between the individual parts of the products, tack on packaging, production equipment and teeth during chewing and to allow easier handling of products and simplify processing. They are used mainly for dehydrated and frozen vegetables (magnesium silicate, E553a), sweets and chewing gum (starch), milk powder, cheeses, pasta (mono- and diacylglycerols, E471) and roasted nuts (starch). To reduce the stickiness of the product to the manufacturing equipment silicone oils and lecithin may be used. Lubrication of sheets for baking pastry is also done with silicone oil.

Sequestrants (chelating agents) form complexes with metal ions, thus preventing oxidation, undesirable discoloration and turbidity (calcium disodium ethylenediaminetetraacetate, E386 and its salts and phosphates, E450–E452).

Synergists and potentiators are substances that increase the effects of other additives. They are used to increase the activity of antioxidants (polyphenols, E452; citric acid, E330), emulsifiers (phosphates, E450), aromatic compounds (sodium hydrogen glutamate, E621; 5′-nucleotides, such as inosine 5′-phosphate, E630).

Packaging gases (other than air), which are introduced into the containers before, during or after filling with the foods have the role of an inert or modified atmosphere (e.g. nitrogen in ground coffee packaging, E941).

Propellants are substances that expel a food from a container or facilitate the formation of foam. For whipped creams and other dairy products, nitrous oxide (E942) is used. In cases where acidic agents can be used, it is possible to use carbon dioxide (E290) and, if foaming is desired, nitrogen (E941) can be used.

Catalysts speed up chemical reactions in which they do not actually enter. They are typically used in small quantities. Raney nickel is used as a catalyst in hydrogenation of oils and sodium methoxide (sodium methanoate) in the transesterification of fats. Acids are used as catalysts in the production of certain modified starches. Catalysts also include the enzymes used as food additives, such as amylase (E1100), proteases (E1101, for example papain, bromelain and ficin), glucose oxidase (E1102), invertase (E1103) and lipases (E1104).

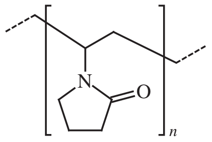
Solvents are additives that allow the extraction of desirable compounds, their dissolution and dilution. They also serve as carriers of aromatic compounds. For the extraction of hops, coffee, tea and spice, hexane, dichloromethane, acetone, trichloroethylene or supercritical carbon dioxide (for the extraction of caffeine from coffee or tea) are most commonly used. Ethanol is used as a solvent of aromatic substances for confectionery; monoacylglycerols are used as solvents for antioxidants and polyols for flavour potentiators.

11.7.4 Legislation

The category of auxiliary agents includes a vast array of food additives. In many cases, it is permissible to use these substances in the amount of *quantum satis* or under good manufacturing practice, but for some commodities maximum levels of additives are set.

Anti-caking agents may be used up to the maximum amounts and only for specified foods. For example, for normal table salt, cocoa powder and potato flakes, silicon dioxide (E551, 20 g/kg) is used as the anti-caking agent. Other agents may be used for coffee and tea whiteners such as silicon dioxide, calcium phosphate (E341), magnesium carbonate (E504) and other agents at levels of 10 g/kg. Talc (E553b) may be added to rice in specified quantities. Dimethylpolysiloxane (E900, **11-78**) may be used as an anti-foaming and anti-caking agent at an amount that does not exceed the specified maximum limits (100 mg/kg in chewing gum and 10 mg/kg in other foods). For the polishing and surface treatment of confectionery, chocolate, walnut kernels, coffee beans and fresh fruits (citrus fruits, melons, apples and pears) some waxes may be used, such as beeswax (E901), candellila wax (E902) and carnauba wax (E903), and shellac (E904). For tableting and coating of tablets, polyvinylpyrrolidone (E1201, **11-79**) and polyvinylpolypyrrolidone (E1202) may be used. For dissolution, dilution and other preparations of additives (colourings, emulsifiers and antioxidants) and flavourings only propane-1,2-diol (propylene glycol, E1520) may be used, the maximum amount of which, except for baby food, is 1000 mg/kg and polyethylene glycol

6000, $HO{-}CH_2{-}(CH_2{-}O{-}CH_2{-})_n{-}CH_2{-}OH$, (E1521). The numbers that are often included in the names of polyethylene glycols indicate their average molecular weights. For antioxidants and fat-soluble pigments, certain salts are allowed as carriers, such as calcium carbonate (E170), calcium acetate (E263), sodium citrates (E331) and numerous others, lecithins (E322), a number of polysaccharides, sugar alcohols and emulsifiers. For food dyes, sorbitan esters (E491–E495) and silicon dioxide (silica, E551) are permitted as emulsifiers. For emulsifiers, colours and flavourings some silicates can be used as anti-caking agents, such as calcium silicate (E552). Beeswax (E901) is only allowed for food dyes. Pyrrolidone polymers polyvinylpyrrolidone (E1201, **11-78**) and polyvinylpolypyrrolidone (E1202), various modified starches and esters, such as triethyl citrate (E1505) and triacetin (E1518), are allowed as stabilisers for sweeteners.



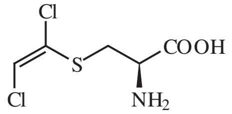
11-79, polyvinylpyrrolidone

11.7.5 Health assessment

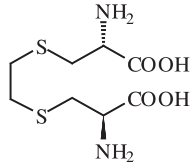
Some food additives are considered as food (such as gelatin, non-fat milk powder, honey, sucrose and starch), and for a number of other food additives ADI values are not specified, for example for various oxides, hydroxides and salts (such as magnesium oxide, silica, calcium chloride and calcium stearate), some polysaccharides (such as carrageenans), glycerol, polyvinylpolypyrrolidone, solvents (ethanol), waxes (beeswax, candilla wax and canauba wax) and others, and no side effects are known. Other substances are considered to be inert (such as dimethyl polysiloxane and polyvinylpolypyrrolidone). Carbon dioxide in concentrations $> 10\%$ by volume is a smothering agent, as is nitrous oxide in high concentrations. Calcium oxide and sodium methoxide are classified as corrosive chemicals.

ADI values are determined for other substances: for phosphoric acid and phosphates (the maximum total intake of phosphorus from phosphates should be 70 mg/kg body weight), calcium D-gluconate and polyvinylpyrrolidone (**11–78**, 50 mg/kg body weight), propane-1,2-diol and ethyl acetate (25 mg/kg body weight) and tannin (0.6 mg/kg body weight). Raney nickel is classified as a possible carcinogen.

Some chlorinated solvents previously used for the extraction of fats, reacted with proteins with the formation of toxic products. For example, soybean meal extracted with trichloroethylene caused, when fed to calves, aplastic anaemia (a condition that occurs when the body does not produce enough new blood cells) caused by toxic (*Z*)- and (*E*)-isomers of *S*-(1,2-dichlorovinyl)-L-cysteine (**11-80**), which arises as a reaction product of protein-bound cysteine with trichloroethylene and subsequent proteolysis. The reaction of 1,2-dichloroethane with proteins in cod fillets did not lead to chlorinated reaction products, but the nutritional value was reduced due to the formation of non-utilisable cysteine, histidine and methionine. Protein-bound cysteine yields, for example, *S*,*S*′-ethylenebiscysteine (**11-81**), which is resistant to proteolysis.



11-80, (*E*)-*S*-(1,2-dichlorovinyl)-L-cysteine



11-81, *S*,*S*′-ethylenebis-L-cysteine

12

Food Contaminants

12.1 Introduction

Food contamination refers to foods that are tainted or spoiled because they contain toxic substances, microorganisms or parasites that make them unsuitable for consumption. Food contamination is a serious issue because it results in food-borne diseases that affect many people worldwide each year. Hence, awareness of potential sources of food contamination is an important component of good nutrition.

Under certain conditions, various substances may arise in food or permeate into food from the external environment that can have a negative impact on human health. Substances that can get into food accidentally during agricultural production (agrochemicals), during technological or culinary processing, as well as during storage, transportation and marketing, or due to environmental pollution, are called food contaminants. Food contaminants also include some toxic secondary metabolites of microorganisms. Food contaminants can be classified according to the source of contamination and the mechanism by which they enter the food product. Important groups of contaminants include:

- **primary** or **exogenous** contaminants originating from external sources

- **secondary** or **endogenous** contaminants, which arise in food due to various physical and chemical factors from natural components of raw materials during food processing operations, better known as **food-borne**, **process-induced**, **processing** or **technological** contaminants.

In principle, the circumstances under which the primary (exogenous) or secondary (endogenous) organic and inorganic contaminants get into the human food chain may be summarised in the following points:

Agricultural production

- use of pesticides (so-called modern pesticides) and plant growth regulators;

- fertilisation (toxic metals, especially cadmium);

- environmental pollution, pollution load, for example the long-range transport (persistent organohalogen compounds) such as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCBs), DDT group of insecticides, polycyclic aromatic hydrocarbons (PAHs) and phthalic acid esters;

- use of surface water irrigation (especially pesticides and their metabolites, industrial chemicals and toxic metals);

- attack by microorganisms, especially by fungi (mycotoxins);

- veterinary treatment (drugs, hormones, pesticides and other compounds).

Storage and processing

- postharvest application of pesticides (mainly organophosphates and pyrethroids);

- formation of toxic degradation products from relatively non-toxic pesticides (ethylenethiourea, *N*,*N*-dimethylhydrazine);

- attack by microorganisms (bacterial toxins and mycotoxins);

- some technological or cooking procedures (heterocyclic amines, acrylamide, furan, 3-chloropropane-1,2-diol and its fatty acid esters, polycyclic aromatic hydrocarbons and nitrosamines);

- penetration of additives from plastics (such as phthalates and bisphenol A).

A number of exogenous and endogenous chemicals (e.g. PAHs, PCBs, PCDD, PCDF polychlorinated hydrocarbons) are classified as **persistent organic pollutants** (POPs), which are organic compounds resistant to environmental degradation through chemical

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and biological processes. POPs are typically hydrophobic and lipophilic substances that partition strongly into solids (notably organic matter) in aquatic systems and soils rather than being dissolved in water. They also partition into lipids in living organisms rather than entering the aqueous milieu of cells, are stored in fatty tissue and accumulate in food chains as their metabolism is slow. The fate of persistent organic pollutants (POPs) in the abiotic (consisting of soil, water and air) and biotic (living organisms) components of the environment are related to their physical and chemical properties, such as high melting and boiling points, high vapour pressure (related to the value of Henry's law constant k_H), high lipophilicity (measured by the logarithm of octanal–water partition coefficient K_{OW}), low water solubility and high tendency for adsorption to soil or sediment particles (measured by the logarithm of adsorption coefficient K_{OC} normalised to the organic carbon content).[1]

Contaminants entering into the organism of biota leads to more or less extensive **biotransformation** and **bioaccumulation**. Biotransformation is the process whereby a substance is changed (transformed) into another by a biochemical reaction within the body. Biotransformation is thus a key body defence mechanism, the relative effectiveness of which depends on several factors, including species, age, gender, nutrition and some others. When biotransformation results in metabolites of lower toxicity, the process is known as **detoxification**. In many cases, however, the metabolites may be more toxic than the parent substance. This process is known as **bioactivation**. Bioaccumulation is the process by which contaminants from all sources combined (such as water, food and air) and through any route, including respiration, ingestion or direct contact with contaminated water or sediments, concentrate in tissues of living organisms (biota). **Bioaccumulation factor** (BAF) is the ratio of the contaminant in an organism to the concentration in the ambient environment at a steady state. **Bioconcentration** is the process of accumulation of water-borne chemicals by fish and other aquatic biota through non-dietary routes. A major distinction between bioaccumulation and bioconcentration is that in bioconcentration no dietary intake is involved. The measure of the tendency for a substance in water to accumulate in tissue of aquatic biota is called the **bioconcentration factor** (BCF), defined as the ratio of a contaminant concentration in biota to its concentration in the surrounding medium (water). **Biomagnification**, also known as bioamplification, is the result of bioaccumulation and biotransfer, by which tissue concentration of a contaminant in organisms at one trophic level exceeds tissue concentration in organisms at the next lower trophic level in the food chain. **Biomagnification factor** (BMF) refers to the ratio of contaminant concentration in biota to that in the surrounding water when the biota was exposed via contaminated food.

Among the most observed chemical contaminants are toxic minerals (metals, metalloids and other inorganic compounds), radioactive isotopes, mycotoxins and other microbial toxins, halogenated organic compounds, phytotoxins as representatives of natural contaminants and others. For the selection of contaminants to be covered in this chapter, a number of criteria were taken into account, the most important of which include:

- acute and chronic toxicity determined in toxicological studies (particularly neurotoxicity, carcinogenicity, mutagenicity, teratogenicity, immunotoxicity, interference with hormonal processes and other types of toxicities);
- frequency at which the given contaminant has been proven to be the cause of poisoning of humans or animals;
- frequent occurrence in foods representing important items of the food basket (special attention is paid to breast milk);
- persistence and frequency of occurrence of the given contaminant in the environment, possible conversion to (bio)products with higher toxicity and ability to bioaccumulate in the human food chain;
- massiveness of inputs (emissions) of the given contaminant into the environment from industry, agriculture, urban areas and other sources;
- importance of foods in which the contaminant occurs in terms of international trade.

There are considerable differences between experts and the general public in the perception of health risks associated with consumption of contaminated food. In a survey conducted in the UK in the early 1990s, the professionals put in first place the risks associated with microbial contamination, followed by risks arising from the presence of natural toxins, pollution from industrial contaminants, residues of veterinary drugs, pesticides and the presence of food additives. The general public considered the primary problem pesticide residues, as well as industrial contaminants, additives (so-called 'E additives'), residues of veterinary drugs, microbial contamination: they considered natural toxins the least serious risk. For various reasons, the concerns of ordinary consumers are frequently unfounded, biased and often become the argument in favour of the preference of organic products (organic food). Legislation and practical measures taken to ensure the hygienic and

[1] Values of Henry's law constant ($k_H = p/c$, where p is the partial pressure of the solute in the gas above the solution and c is the concentration of the solute) is a quantity frequently applied in the thermodynamic description of dilute aqueous solutions, which is used in environmental chemistry and atmospheric physics as a major criterion for describing air–water partitioning of solutes at near ambient conditions. It plays a major role in evaluating the transport of pollutants between atmosphere and aquatic systems, rainwater and aerosols. The octanol–water partition coefficient (K_{OW}) is a dimensionless number defined as the ratio of the compound's concentration in a known volume of octan-1-ol (c_O) to its concentration in a known volume of water (c_W) after the octan-1-ol and water have reached equilibrium. It has been found to be related to water solubility, soil/sediment absorption coefficients and bioconcentration factors of pollutants for aquatic life. The adsorption coefficient normalised to the organic carbon content of the soil (sediment) K_{oc} is a useful indicator of the binding capacity of a chemical on organic matter of soil and sediments and allows comparisons to be made between different chemicals. For example, $K_{oc} = c_{soil}/c_{aq} = c_{sediment}/c_{aq}$ where $= c_{soil}$ ($c_{sediment}$) are concentrations of test substance in soil (sediment) in equilibrium and c_{aq} = concentration of test substance in aqueous phase in equilibrium.

toxicological quality of food are applied to contaminants, natural toxins and food additives in the same way.

If the acquired knowledge and available data allow the estimation of health risks, then appropriate measures are incorporated into legislation. This is an important tool to protect the health of consumers, while allowing participants in the process of food production and distribution to set up appropriate conditions which lead to the reduction or elimination of contamination. In this context, episodic crisis should be noted, when the food contains toxic substances which were until then unknown. In many cases the aim of their use is food adulteration. Examples include carcinogenic synthetic Sudan pigments, which have been used frequently to colour spices, such as 1-(2-methoxyphenylazo)-2-naphthol, known as Sudan Red G, as an adulterant in turmeric, or 1-phenylazonaphth-2-ol (Sudan I, also known as Solvent Yellow 14 or Solvent Orange R) found in the United Kingdom in Worcestershire sauce produced by Premier Foods contaminated by adulterated chilli powder (this problem peaked in 2004–2007), or melamine (see Section 12.8.3) used mainly in China in order to increase the apparent protein content in food products including infant formula and milk powder (the scandal broke in 2007).[2] The presence of melamine was then reported in a wide range of products, where the contaminated milk was used as an ingredient.

The control system of residues of contaminants generally takes into account the toxicity of the given compound, the method of its use, the possibility of exposure of humans or livestock to residues and indicators of consumption of potentially contaminated foods. At the international level, the relevant recommendations are formulated by the commission of the Codex Alimentarius Committee and by the International Technical Consultation for Veterinary Drugs Registration. The challenge now is to achieve international harmonisation of criteria, in particular generally accepted maximum residue limits (MRLs).

Ongoing evaluation of the toxicity of pesticides is attended by experts of the Joint Meeting on Pesticide Residues (JMPR) representing FAO and WHO. This body effectively cooperates with the Codex Committee on Pesticide Residues (CCPR) and publishes consensus MRL data, which serve as a basis for the adoption of legislative measures at the international level. At the same time, the CCPR compiles a list of particularly hazardous pesticides, which should be the subject of subsequent JMPR evaluations.

The institution responsible for the common (supranational) registration of veterinary drugs in the EU is the Committee for Veterinary Medicinal Products (CVMP), which is organisationally subordinate to the European Medicines Evaluation Agency (EMEA). The basic principle is that no new veterinary preparation can be registered for use if the manufacturer cannot demonstrate that the drug is not safe to the treated animal (patient), the consumer, the attending person and the environment.

[2]The United Nations' food standards body, Codex Alimentarius Commission, has set the maximum amount of melamine allowed in powdered infant formula to 1 mg/kg and the amount of the chemical allowed in other foods and animal feed to 2.5 mg/kg. The United States does not allow melamine to be used as a food additive.

Major international activity focused on the issue of food contaminants also comes from the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food), founded by the WHO in 1976 in order to inform governments of the participating countries, the Codex Alimentarius Committee, other interested institutions and the public about typical concentrations of contaminants in foods and their contribution to human exposure. The programme provides globally collected data on the occurrence of selected toxic metals (e.g. lead, cadmium and mercury), pesticides (such as DDT, aldrin and dieldrin), polychlorinated biphenyls (PCBs) and mycotoxins (aflatoxins). In addition to issuing the reports, another aim of the project is to support national monitoring programme projects.

Maximum limits for certain contaminants (nitrates, some toxic trace elements, 3-chloropropane-1,2-diol, polycyclic aromatic hydrocarbons, selected mycotoxins and polychlorinated biphenyls and dioxins) in foods (usually related to the edible part), valid for all EU Member States, are set by Commission Regulation (EC) No. 1881/2006. Products containing contaminants exceeding the maximum levels should not be placed on the market as such either, after mixture with other foodstuffs or used as ingredients in other foods. Foods containing mycotoxins cannot be deliberately detoxified by chemical treatment. Maximum levels of contaminants should always be set at a level that is reasonably achievable by following good agricultural and manufacturing practices. Food business operators have to apply measures to prevent and reduce the contamination as far as possible in order to protect public health, especially of infants and young children.

This chapter is divided into seven main sections. The first of these sections is focused on technological contaminants, namely heterocyclic amines, acrylamide, furan, chloropropanols and their fatty acid esters, polycyclic aromatic hydrocarbons, monocyclic aromatic hydrocarbons, nitroso compounds, and ethyl carbamate. Other sections deal with microbial toxins (mycotoxins and bacterial toxins), persistent organohalogen contaminants (such as polychlorinated biphenyls, dibenzodioxins and dibenzofurans), chlorinated aliphatic hydrocarbons, pesticides (persistent chlorinated hydrocarbons and modern pesticides), veterinary medicines and contaminants from packaging materials. Presented for each of these contaminants are structures, properties, occurrence and the main sources of dietary intake, mechanisms of formation, possibilities of food contamination, prevention and mitigation and health and toxicological evaluations.

12.2 Technological contaminants

Toxic or potentially unsafe compounds can arise even in industrial and culinary processing of common food raw materials and foods from their natural components. One of the oldest ways to ensure the microbiological safety of foods, increase their shelf life and also achieve the desired organoleptic properties (smell, taste, colour and texture) is heat treatment, by methods including smoking. Processes operating at elevated temperatures during various industrial operations or culinary processing induce, depending on the

specific physical conditions and chemical composition, a variety of enzymatic (biochemical) and non-enzymatic (chemical) reactions leading to the formation of new compounds. Many compounds produced in this way have antioxidant and antimutagenic properties, but industrial operations or culinary processing may also lead to the formation of compounds with proven toxic potential, and in some cases these compounds may show mutagenicity and carcinogenicity. This group of contaminants are mostly considered as processing or technological contaminants.

Precursors of these compounds are common amino acids or proteins, reducing sugars (including ascorbic acid), fatty acids, triacylglycerols and other lipids, while some other food components may also be precursors of certain technological contaminants. Often there are multiple precursors. Some additives may also participate in the formation of these contaminants, such as hydrochloric acid (known as protein hydrolysing agent in the production of acid protein hydrolysates and in technological practice considered to be an ancillary material), chlorine (chlorine dioxide) from drinking water and nitrites (used as antimicrobial agents and stabilisers of meat colour).

An example of processing contaminants arising at higher temperatures, which decades ago were considered risky in terms of health effects, are primary aromatic amines (aminoimidazoazaarenes), whose precursor is the amino acid creatinine, Strecker aldehydes and heterocyclic nitrogen compounds arising in the Maillard reaction. Some other amino acids and proteins may also be precursors of aminoimidazoazaarenes.

The list of intensively studied chemical contaminants generated by the Maillard reaction at the beginning of the 21st century has spread to acrylamide and furan. Acrylamide is formed from the amino acid asparagine in reactions with reducing sugars, again in the Maillard reaction. Furan precursors may include some amino acids, carbohydrates (including ascorbic acid), unsaturated fatty acids and other compounds, such as carotenoids. Chloropropanols and their esters with higher fatty acids, in particular 3-chloropropane-1,2-diol and its esters, are another group of processing contaminants. Their precursors in many foods are lipids (especially triacylglycerols, phospholipids and their hydrolysis products) and naturally present or intentionally added sodium chloride (salt). In hydrolysed vegetable proteins, the precursors of chloropropanols are lipids and hydrochloric acid. Nitroso compounds, in particular nitrosamines, are formed from nitrites (and nitrogen oxides) by reaction with amines and amino acids containing secondary amino groups. Precursors of nitrosamines may also become their transformation products and reaction products of the Maillard reaction (glycosylamines, aminodeoxysugars and nitrogen heterocycles), some phospholipids and some other nitrogen-containing substances. Polycyclic aromatic hydrocarbons are formed by pyrolysis of a number of compounds, such as steroids and aromatic hydrocarbons, as well as by pyrolysis of basic nutrients. Ethyl carbamate (urethane) is formed from natural precursors during fermentation processes used in the production of certain spirits and other products. The precursors are cyanides derived from cyanogenic glycosides, the amino acid citrulline, carbamoyl phosphate (an intermediate of the biosynthesis of pyrimidines) and urea or the previously used preservative diethyl dicarbonate. During fermentation processes, biogenic amines are also formed as byproducts of decarboxylation of amino acids (see Section 10.3.3.10).

Some processing contaminants may also be simultaneously classified as exogenous contaminants, but exogenous sources are minimal in comparison with endogenous sources. For example, the exogenous source of polycyclic aromatic hydrocarbons in the food chain may be a contaminated environment or tobacco smoke. Tobacco smoke can also be a source of nitrosamines. Some contaminants classified as exogenous may also arise during food processing in smaller amounts. Typical examples of such contaminants are monocyclic aromatic hydrocarbons, such as benzene, which is produced by decarboxylation of benzoic acid, and styrene, which is a product of cinnamic acid decarboxylation.

A number of other compounds that can be classified as technological contaminants are covered elsewhere in this book, in chapters devoted to proteins, lipids and carbohydrates. For example, thermal processes in alkaline media give rise to unusual amino acids from the proteins, such as lysinoalanine (see Section 2.5.1.3.4) and D-isomers of amino acids (see Section 2.5.1.2), the Maillard reaction gives rise to potentially toxic (mutagenic) compounds, such as methylglyoxal (see Section 4.7.1.2.2) and 5-hydroxymethylfuran-2-carbaldehyde (see Section 4.7.1.1.3), which are formed as transformation products of sugars. Other toxic products may be formed from lipids. Examples of compounds resulting from the oxidation of lipids are acrolein (see Section 3.8.2.2.3), malondialdehyde and 4-hydroxynon-2-enal. Also discussed is the health hazard of transformed glycosylated proteins, known by the acronym AGE, produced in the advanced stages of the Maillard reaction or the products of the subsequent oxidation of lipids (lipoxidation) known as ALE (see Section 3.8.1.12.1).

The following sections deal in detail only with processing contaminants whose occurrence (and amount) in foods is either regulated by EU legislation (for which MRL are set), or with processing contaminants the evaluation of which is subject to various monitoring studies. The aim of these studies is to collect sufficient information to estimate dietary exposure to these substances and evaluate the associated health risks.

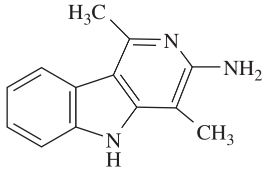
12.2.1 Heterocyclic amines

12.2.1.1 Structure and nomenclature

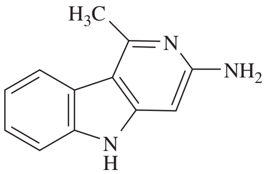
The study of toxic products of the Maillard reaction was initiated in 1977 with the finding that the mutagenicity of charred grilled meat and fish is produced, in addition to neutral polycyclic aromatic hydrocarbons, such as benzo[*a*]pyrene, by certain basic compounds. Later research has shown that the basic compounds are mainly primary heterocyclic amines. These substances are divided into two groups:

- non-IQ mutagens
- IQ mutagens.

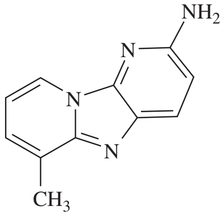
Non-IQ (also known as aminocarbolines or pyrolytic heterocyclic aromatic amines) and IQ mutagens are typical technological contaminants. More than 20 mutagenic heterocyclic aromatic amines have been isolated from various cooked meats, fish and poultry. Mutagens of the first group are mainly **pyridoimidazoles** and **pyridoindoles**. These substances were first isolated from pyrolysates of amino acids and proteins obtained at temperatures of 300–800 °C. Particularly high mutagenicity is shown by pyrolysates of tryptophan, glutamic acid, lysine, ornithine and phenylalanine and pyrolysates of some proteins (casein, wheat gluten and soy globulin). It was subsequently found that these substances likewise arise during thermal processing of foods, such as roasting, pan frying, grilling or grilling directly over an open flame. The first group of mutagens includes 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole, also known as Trp-P-1 (**12-1**), which was found in tryptophan pyrolysates together with 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2, **12-2**), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole arising from glutamic acid (Glu-P-1, **12-3**) and further 2-aminodipyrido [1,2-*a*:3′,2′-*d*]imidazole (Glu-P-2, **12-4**), 3,4-cyclopentenopyrido[3,2-*a*]carbazole for lysine pyrolysates (Lys-P-1, **12-5**), 4-amino-6-methyl-1*H*-2, 5,10,10*b*-tetraazafluoranthene from ornithine pyrolysates (Orn-P-1, **12-6**), 2-amino-5-phenylpyridine (Phe-P-1, **12-7**), 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC, **12-8**) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC, **12-8**).



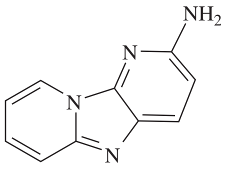
12-1, Trp-P-1



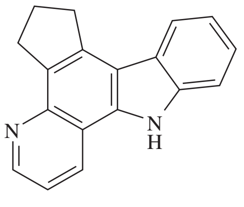
12-2, Trp-P-2



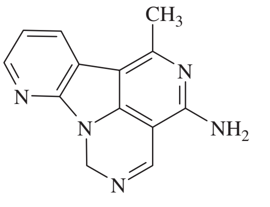
12-3, Glu-P-1



12-4, Glu-P-2

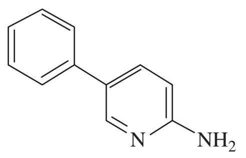


12-5, Lys-P-1

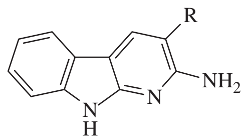


12-6, Orn-P-1

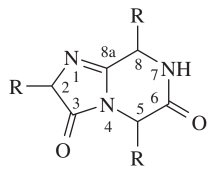
Pyrolysates of aliphatic amino acids, such as alanine, valine, leucine and isoleucine (obtained at 230–250 °C) contained, in addition to the corresponding 2,5-dioxopiperazines (see Section 2.5.1.3.3), compounds of similar structures called **imidazopyrazinediones** (**12-9**) that are formed in the reaction of 2,5-dioxopiperazines with another molecule of amino acid and subsequent cyclisation. **Diimidazopyrazinediones** (**12-10**) are formed analogously. These compounds do not show toxic effects as they are not primary aromatic amines.



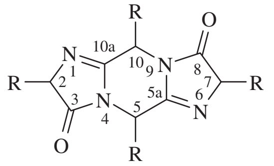
1**2-7**, Phe-P-1



12-8, AαC, R = H
MeAαC, R = CH_3



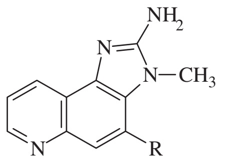
12-9, 7,8-dihydro-2*H*-imidazo [1,2-*a*]pyrazine-3,6-dione



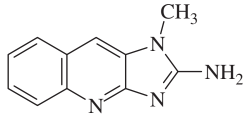
12-10, 2,5,7,10-tetrahydrodiimidazo [1,2-*a*:1′,2′-*d*]pyrazine-3,6-dione

Mutagens of the second and most important group include **aminoimidazoquinolines**, **aminoimidazoquinoxalines** and **aminoimidazopyridines**, namely 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, **12-11**), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ or 4-MeIQ, **12-11**), 2-amino-3,4-dimethylimidazo[4,5-*b*]quinoline known as IQ[4,5-*b*] (**12-12**), 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx, **12-13**), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (4-MeIQx, **12-13**), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx or 8-MeIQx, **12-13**), 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (4,8-DiMeIQx, 12-13), 2-amino-3,7,8-trimethylimidazo-[4,5-*f*]quinoxaline (7,8-DiMeIQx, **12-13**), 2-amino-1-methylimidazo[4,5-*g*]quinoxaline (IgQx, **12-14**), 2-amino-1,7-dimethyl imidazo[4,5-*g*]quinoxaline (7-MeIgQx, 12-14), 2-amino-1,6,7-tri methylimidazo[4,5-*g*]quinoxaline (6,7-DiMeIgQx, **12-14**), **2**-amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline (7,9-DiMeIgQx, **12-14**), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP, **12-15**), 2-amino-1-methyl-6-(4-hydroxyphenyl)imidazo-[4,5-*b*]pyridine (4′-OH-PhIP, **12-15**), IFP, 2-amino-(1,6-dimethylfuro[3,2-e]imidazo[4,5-b])pyridine (**12-16**), 2-amino-dimethylimidazopyridine (DMIP, **12-17**) and 2-aminotrimethylimidazopyridine (TMIP, **12-18**). All these IQ mutagens containing imidazole cycle derived from creatine/creatinine and an amino group in the C-2 position of the cycle are often collectively called **aminoimidazoazaarenes**. The remaining parts of their molecules

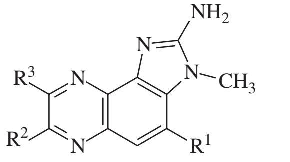
are formed from the Maillard reaction products. Aminoimidazoquinoline (IQ) and aminomethylimidazoquinoline (MeIQ) were first isolated from broiled sun-dried sardines by Japanese scientists in 1980 and one year later aminomethylimidazoquinoxaline (MeIQx) was also isolated from fried beef.



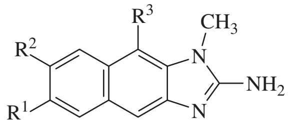
12-11, IQ, R = H
4-MeIQ, R = CH_3



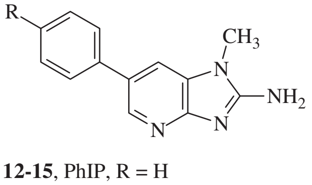
12-12, IQ[4,5-*b*]



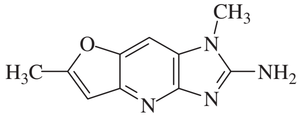
12-13, IQx
4-MeIQx, $R^1 = CH_3$, $R^2 = R^3 = H$
8-MeIQx, $R^1 = R^2 = H$, $R^3 = CH_3$
4,8-DiMeIQx, $R^1 = R^3 = CH_3$, $R^2 = H$
7,8-DiMeIQx, $R^1 = H$, $R^2 = R^3 = H$



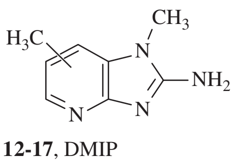
12-14, IgQx, $R^1 = R^2 = R^3 = H$
7-MeIgQx, $R^1 = R^3 = H$, $R^2 = CH_3$
6,7-DiMeIgQx, $R^1 = R^2 = CH_3$, $R^3 = H$
7,9-DiMeIgQx, $R^1 = H$, $R^2 = R^3 = CH_3$



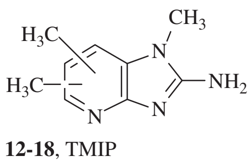
12-15, PhIP, R = H
4´-PhIP, R = OH



12-16, IFP



12-17, DMIP



12-18, TMIP

12.2.1.2 Occurrence, main sources and dietary intake

The exposure to aminoimidazoazaarenes primarily occurs through meat and fish, but small amounts of these compounds may also be present in flavourings, beer, wine and cigarette smoke. The exposure can similarly occur by inhalation of aerosol particles generated during cooking or that are present in the combustion gas, especially in diesel engine exhaust gas. In foods, PhIP is found in the highest levels, followed by MeIQx, MeIQ and IQ. Their total amount in thermally processed meat varies by meat type, cooking method and 'doneness' level (rare, medium or well done) and usually ranges from <1 to about 500 μg/kg and the current average concentrations are lower than 100 μg/kg. Total daily intake of aminoimidazoazaarenes is within the limits of 160–1800 ng and the dietary exposure is estimated to be 1–17 ng/kg body weight per day.

PhIP is the main aromatic amine formed in very well done barbequed chicken (up to 305 μg/kg), oven-broiled bacon (16 μg/kg), pan-fried bacon (4.9 μg/kg), oven-broiled ground beef (0.04–2.9 μg/kg), pan-fried beef steak (0.04–12.46 μg/kg). The amount of PhIP in meat depends greatly on its type and the method of heat treatment. Available data vary widely. For example, salmon cooked in a pan, in a convection oven or grilled at 200 °C may contain PhIP at the level of 0.02–2.2 μg/kg and in extreme cases even 73 μg/kg. PhIP has also been identified in flavourings, beer and wine (0.01–480 μg/kg), cigarette smoke, in the air and surface waters. Daily intake of PhIP varies from 286 to 458 ng, and its dietary exposure is estimated at 17 ng/kg body weight. Cooked meats also contain 4′-hydroxy analogue of PhIP, 4′-OH-PhIP. For example, the highest content of 4′-OH-PhIP was found in fried and griddled chicken breast, the concentration being 43.7 and 13.4 μg/kg, respectively, whereas the corresponding PhIP concentrations were 19.2 and 5.8 μg/kg. MeIQx was found in beef, pork and chicken meat and in fish. The highest amount in beef (steaks) reached 8.2 μg/kg, in hamburgers 4.6 μg/kg, but in pork the concentrations of MeIQx are relatively low (except for bacon, 0.9–27 μg/kg). Concentrations in grilled chicken and in chicken roasted in the oven or in a pan were 9 μg/kg, and in fish about 1.2 μg/kg. MeIQx was also found in thickened meat broth, wine, surface water and air. Its daily intake is estimated at 33–44.8 ng and dietary exposure to 2.61 ng/kg body weight.

MeIQ occurs less frequently and at lower concentrations as compared with other aminoimidazoazaarenes. The highest concentrations (0.03–72 μg/kg) have been found in fish, at the upper limit in grilled sardines and at the lower limit in baked fish. It is found in low or undetectable quantities in beef, pork (0.02 μg/kg in roasted meat, 1.7 μg/kg in bacon) and chicken. MeIQ was also identified in thickened meat broth, roasted coffee beans and cigarette smoke. Dietary exposure is estimated at 0.6 ng/kg body weight.

IQ was first found in grilled fish, fried minced beef and beef extracts, and later in many other foods (steaks, roasted chicken, fried minced pork and fried eggs) and cigarette smoke. Its concentrations range from <0.1 to >150 μg/kg, but usually do not exceed 1 μg/kg. For example, its concentrations in oven-broiled ground beef range from 0.01 to 0.1 μg/kg and in barbecued chicken 0.02–0.91 μg/kg.

Dietary exposure to IQ is estimated to be 0.28 ng/kg body weight per day.

The other aromatic amines are generally found in variable amounts. For example, well done pan-fried beef steak may contain 23.7 μg/kg 7-MeIgQx, 6.31 μg/kg 7,9-DiMeIgQx, 4.03 μg/kg IgQx and IFP (well-done meats may contain IFP at levels of from 1.4 to 46 μg/kg, 2.06 μg/kg 4,8-DiMeIQx, 0.68 μg/kg 6,7-DiMeIQx and 0.16 μg/kg IQ[4,5-*b*], while AαC and MeAαC are not present.

12.2.1.3 Formation

Heterocyclic amines known as aminoimidazoazaarenes are formed during thermal processing of meat of warm-blooded animals, poultry and fish by reactions of creatine/creatinine (**2-118**) with certain products of the Maillard reaction, but detailed mechanisms of their formation are not yet fully understood. Creatine was postulated to form the 2-amino-3-methylimidazo (2-aminoimidazo) moiety of aminoimidazoazaarenes by cyclisation and water elimination, a reaction that takes place spontaneously at temperatures above 100 °C. This part of the molecule (especially its 2-amino group) is a common moiety of all aminoimidazoazaarenes and is also responsible for their mutagenicity. The remaining parts of their molecules are assumed to arise from the Maillard reaction between sugars and amino acids, which produces various reactive intermediates for the formation of pyridines and pyrazines, such as glycolaldehyde and glyoxal imines, pyridinium cation, pyrazinium cation radical and pyrazinium dication. Aldol condensation of Strecker aldehydes is believed to link the two parts of aminoimidazoazaarenes together (Figure 12.1).

During cooking, heterocyclic amines can react with proteins (and free amino acids) by condensation of the amino groups of heterocyclic amines (such as PhIP) and carboxyl groups of proteins yielding the corresponding amides. The presence of such adducts may be an important factor in evaluating the carcinogenic risk of heterocyclic amines, because a portion of these mutagens formed by cooking change into non-mutagenic adducts. However, the parent heterocyclic amines can be released from high molecular weight compounds by acid hydrolysis or proteolytic digestion in the gastrointestinal tract and may influence human exposure to heterocyclic amines and carcinogenic risk.

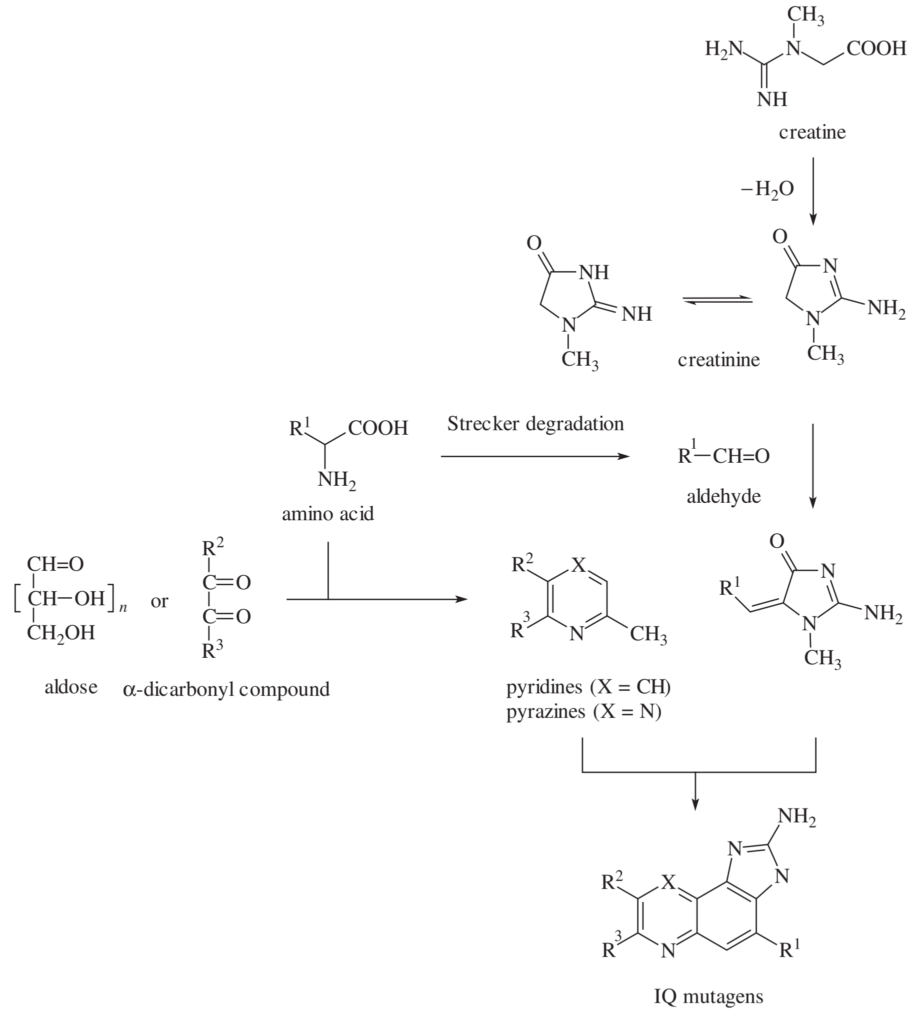


Figure 12.1 Formation of aminoimidazoazaarenes.

12.2.1.4 Health and toxicological assessment

Four compounds show high specific mutagenicity and carcinogenicity, namely 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ, **12-11**), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (4-MeIQ, **12-11**), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (4-MeIQx, **12-13**) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP, **12-15**). In comparison with known mutagens and carcinogens, such as benzo[*a*]pyrene, these compounds cause more serious DNA damage and induce chromosome aberrations (changes in structure and number). They are easily absorbed and distributed in the tissues. IQ mutagens are metabolised by the phase I enzymes (activation by *N*-hydroxylation) and by the phase II enzymes (further activation by the formation of arylnitrenium ions and conjugation with DNA). DNA adducts have been detected in various tissues, such as from the colon and prostate. The risk of carcinogenesis from the intake of individual aminoimidazoazaarenes is difficult to estimate, because there are always mixtures of several compounds in meat, along with other contaminants (polycyclic aromatic hydrocarbons, nitrosamines and other carcinogens).

12.2.1.5 Mitigation

The content of aminoimidazoazaarenes in foods is highly dependent on the particular temperature and time of heat treatment and also on the water content, pH and concentration of precursors. Higher temperatures and longer processing times generally result in higher quantities of aminoimidazoazaarenes. The contents of these contaminants also depend on the type of heat treatment. Direct heating operations or operations with efficient heat transfer, such as grilling, produce higher amounts of aminoimidazoazaarenes than indirect methods, such as cooking in water or steaming. The highest amount of aminoimidazoazaarenes is therefore formed in the surface layers of food exposed to higher temperatures (especially during grilling and roasting) that have lower water content. During cooking in water, these mutagens are either not formed or arise in low quantities. Their concentrations in commercially prepared foods are therefore very low.

The formation of heterocyclic amines of the IQ type is effectively inhibited by antioxidants. The presence of phenolic compounds such as flavanones and flavan-3-ols significantly inhibits their formation. The mechanism of the inhibitory effect is partly based on the elimination of Strecker aldehydes, precursors of IQ mutagens, by condensation with phenols. For example, phenylacetaldehyde, a decarboxylation product of phenylalanine and a precursor of PhIP, produces two adducts, (*E*)-8-(phenylethenyl)naringenin and (*E*)-6-(phenylethenyl)naringenin with naringenin (Figure 12.2).

12.2.2 Acrylamide

12.2.2.1 Structure and nomenclature

Acrylamide, also known as acrylic acid amide or prop-2-enamide (**12-19**) occurs in a wide range of fried, roasted, baked or toasted foods, especially in foods prepared from raw materials of plant

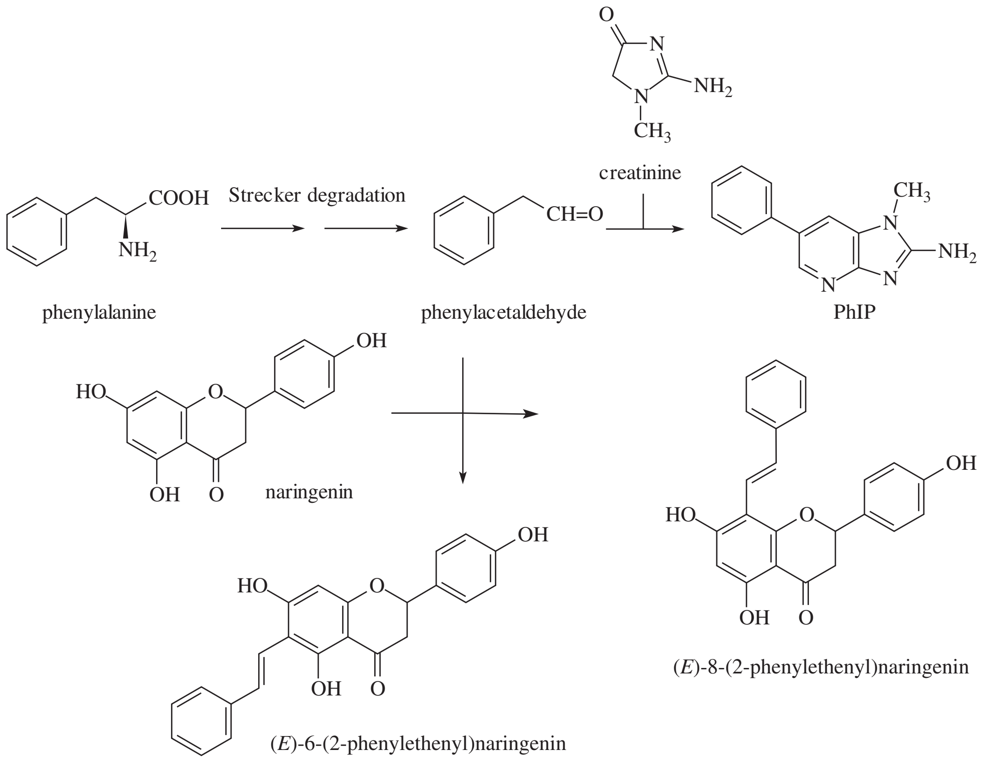
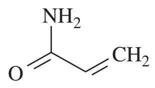


Figure 12.2 Inhibition of PhIP formation by naringenin.



12-19, acrylamide

origin and rich in starch. Although acrylamide has long been a normal component of the human diet, the identification of this contaminant in foods by Swedish scientists in 2002 was a surprise for professionals. This discovery of acrylamide in food is primarily associated with an accident that occurred in Sweden during the construction of a railway tunnel. Dissemination of synthetic acrylamide into the environment due to a technical error induced acute neurotoxic symptoms in construction workers. Livestock and other animals in the area were also affected and surface water contamination led to the death of huge numbers of fish. In the extensive investigations that followed, concentrations of the exposure marker were monitored, which is an acrylamide–haemoglobin adduct. The surprising findings were that there were relatively high levels of that biomarker in unexposed persons from the reference remote locations, which led to identification of thermally processed foods with high starch content as the acrylamide source. Until then, the primary known potential sources of acrylamide had been drinking water and tobacco smoke.

New findings about the presence of this substance in a number of food items, inevitably provoked intense international research, aimed not only to clarify the mechanism of acrylamide formation and evaluation of the health risks from dietary exposure for consumers, but also focusing on finding appropriate strategies to minimise food contamination.

12.2.2.2 Occurrence, main sources and dietary intake

Based on the evaluation of an extensive set of available data, it is assumed that the dietary exposure of western populations to acrylamide is mainly contributed to by fried potato chips (16–30%), potato crisps (6–46%), coffee (13–39%), pastry and sweet biscuits (10–20%), bread and crisp bread (10–30%) and to a lesser extent other foods (<10%). Significant differences in contributions to the total intake of acrylamide depend on the composition of the food basket in different countries. For example in Sweden, the proportion of coffee in the total exposure is estimated at 39%, while in the Netherlands the level is only 13%. In the United States 35% of exposure is attributed to fried potato products, but only 7% to coffee. Exposure to acrylamide in Central Europe is mainly associated with the consumption of fried and roasted potato products.

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the daily intake of acrylamide for the general population is estimated to be between 0.3 and 2.0 μg/kg body weight. It is expected that the daily intake of acrylamide in children may be two to three times higher than in adults. The average daily intake for the general population is estimated at 1 μg/kg body weight. For populations with a high intake of acrylamide and for children, the average daily intake is estimated at 4 μg/kg body weight.

Table 12.1 presents summary data collected in the monitoring implemented in 2002–2008 in EU Member States. The database is managed by the European Commission – Joint Research Centre, Institute for Reference Materials and Measurements. Concentrations of acrylamide fluctuate over a wide range, depending on the raw materials, recipes and methods of culinary or industrial processing. Raw or cooked foods (if temperatures do not exceed 100 °C) usually do not contain acrylamide at all, or contain only very small quantities. Surprisingly high concentrations were found in some types of dried fruits, such as pears and plums, where the concentrations of acrylamide were more than 1000 μg/kg, although the processing temperatures did not exceed 80 °C.

12.2.2.3 Formation

Acrylamide is formed primarily in the Maillard reaction, where the key precursors are the amino acid asparagine, from which is derived the acrylamide skeleton, reducing sugars and various carbonyl compounds resulting from sugar degradation and lipid oxidation that enable the decarboxylation of asparagine. In the absence of reducing sugars, α-hydroxycarbonyl, α-dicarbonyl and other active compounds, only deamination of asparagine proceeds, producing fumaric acid monoamide known as fumaramic acid. The general reaction scheme of acrylamide formation in foods,

Table 12.1 Typical concentrations of acrylamide in various foods in the European market.[a]

Food	Mean value (μg/kg)	Maximum (μg/kg)	Food	Mean value (μg/kg)	Maximum (μg/kg)
Cookies	313–317	4200	Potato chips	348–350	2668
Bread	126–136	2430	Potato crisps	626–628	4180
Cereal breakfast	135–156	1600	Homemade potato products	310–319	2175
Baby cereal nutrition	52–74	353	Canned baby food	23–44	162
Coffee	249–253	1158	Other products[b]	305–313	4700

[a] Data collected according to the recommendation of the European Commission 2007/331/EC.

[b] Pizza, pancakes, waffles, fish fingers, meat balls, chicken nuggets, fried fish, vegetarian steak and roasted cauliflower.

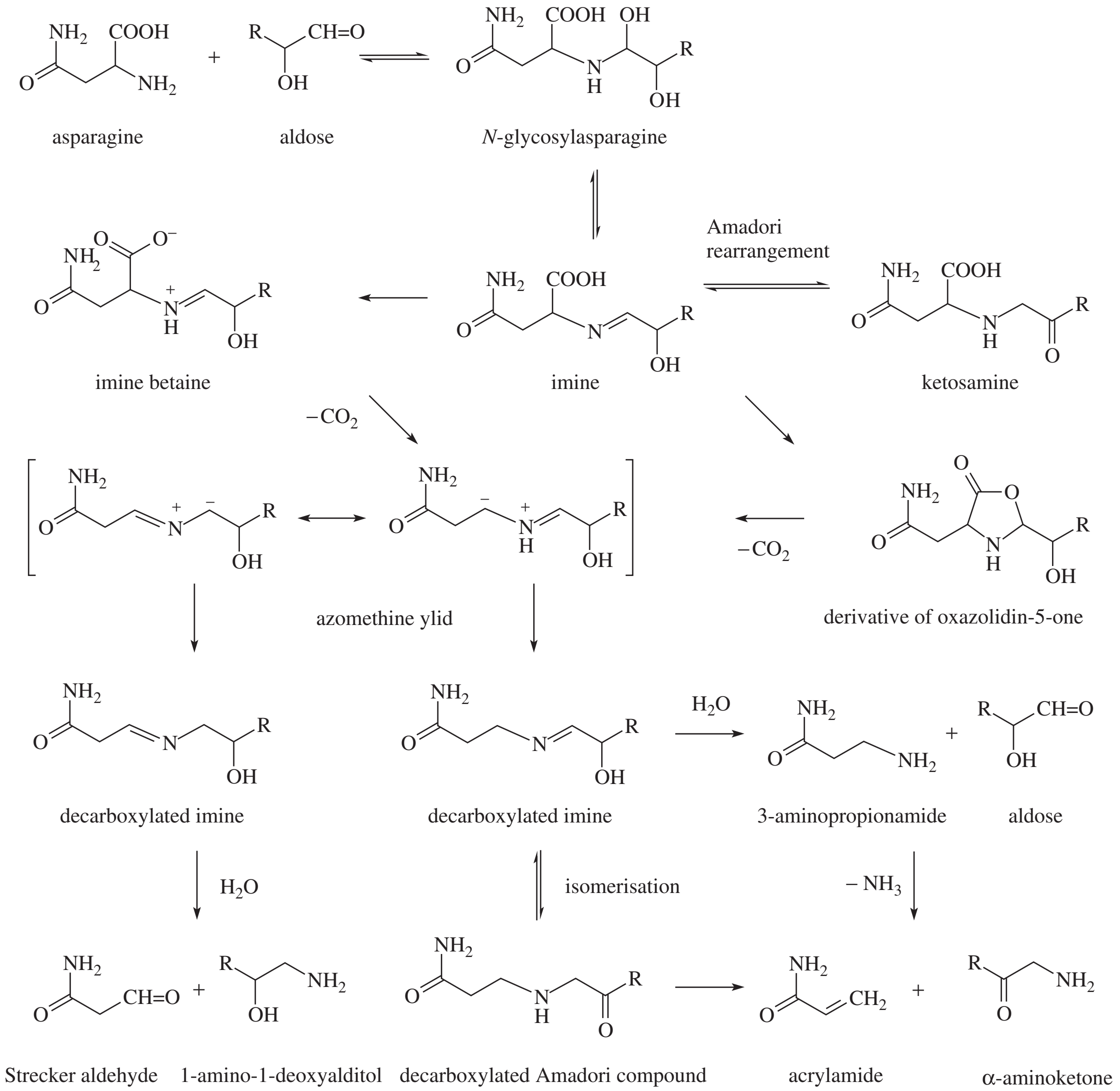


Figure 12.3 Mechanism of acrylamide formation in the presence of α-hydroxycarbonyl compounds.

reflecting current knowledge, is shown in Figure 12.3. A primary product of the reaction is carbinolamine *N*-glycosylasparagine, which dehydrates to form the corresponding imine. In media with low water content, *N*-glycosylasparagine and imine are relatively stable, but in aqueous media imine is hydrolysed or may be rearranged (Amadori rearrangement) to ketosamine (oxoform of 1-amino-1-deoxysugar). Ketosamine is not too significant as a precursor of acrylamide as it is degraded to produce numerous compounds involved in the formation of coloured and flavour-active products in media of low water content.

Decarboxylation of imines derived from α-hydroxycarbonyl compounds, having a free hydroxyl group in the β-position relative to the nitrogen atom, yields the corresponding azomethine ylid. Another alternative of the imine conversion is the formation of imine betaine and intramolecular cyclisation of the product to an oxazolidin-5-one derivative. Both of these compounds provide by decarboxylation azomethine ylid, which can exist in two forms stabilised by resonance that differ in the position of the C=N bond. Both forms of azomethine ylid yield the corresponding imine by decarboxylation. The imine with a double bond between nitrogen and carbon coming from the sugar may be hydrolysed to the parent sugar and 3-aminopropionamide, but may also isomerise to produce a decarboxylated Amadori compound. Acrylamide is then formed, together with an aminoketone, by cleavage of the covalent bond between carbon and nitrogen. The imine double bond between nitrogen and carbon from the asparagine can only be hydrolysed to the Strecker aldehyde of asparagine and 1-amino-1-deoxyalditol, but the decarboxylated Amadori compound does not arise in this case. Another portion of acrylamide may be produced by enzymatic deamination of 3-aminopropionamide.

The amount of acrylamide formed in the reaction mixtures containing α-hydroxycarbonyl compounds, such as glucose, fructose or hydroxyacetone (acetol), is generally much higher than in the case of α-dicarbonyl compounds represented, for example, by butane-2,3-dione (biacetyl) or 2-oxopropanal (methylglyoxal) and other substances. It should also be noted that trace amounts of

acrylamide may result from precursors other than asparagine, such as acrolein and acrylic acid. The thermal degradation of gluten in the production of wheat bread may also give rise to marginal amounts of acrylamide.

12.2.2.4 Health and toxicological assessment

Acrylamide is a neurotoxic compound with genotoxic potential, and according to the IARC (International Agency for Research on Cancer) is classified as a potential human carcinogen (Group 2A).[3] In the organism, acrylamide is rapidly absorbed and evenly distributed. The primary metabolites are glutathione conjugates and the oxidation product glycidamide. Acrylamide and glycidamide can react (form adducts) with macromolecules such as haemoglobin or DNA (Figure 12.4). It is mainly glycidamide that is responsible for the carcinogenic and genotoxic effects, which is, thanks to its epoxy group, much more reactive. The determination of acrylamide adducts with haemoglobin is used in practice to estimate acrylamide exposure. Glycidamide may also be formed by oxidation of acrylamide by fatty acid hydroperoxides.

Current scientific knowledge and epidemiological data have not clearly assessed the cancer risk that may be due to dietary exposure to acrylamide in the general population. An EFSA (European Food Safety Authority) panel on contaminants in the food chain (CONTAM) dealing with various contaminants, confirmed the JECFA 2005 conclusions that consider the acrylamide intake by foods as relatively low. There was an emphasis, however, on the need for ongoing data collection and retrieval of data for further evaluation. The European Commission has not yet set limits for acrylamide, but has issued a recommendation (2007/331/EC) on the monitoring of acrylamide levels in food. To ensure the comparability of analytical results, the chosen methods have to achieve limits of quantification at levels of 30 μg/kg for bread and foods for infants, and 50 μg/kg for potato products, cereal products and coffee.

[3]The International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO), recognises the following groups of carcinogenic substances: Group 1 – carcinogenic to humans, Group 2A – probably carcinogenic to humans, Group 2B – possibly carcinogenic to humans, Group 3 – unclassifiable as to carcinogenicity in humans and Group 4 – probably not carcinogenic to humans.

12.2.2.5 Mitigation

Acrylamide is an unstable compound and may undergo, for example, the addition of nucleophilic amino or sulfhydryl groups of amino acids and other nucleophiles (Michael addition), which can cause its elimination. Therefore, the reported amounts of acrylamide are the result of simultaneous formation and elimination reactions. Outputs of extensive studies aimed at elucidating the mechanisms of acrylamide formation in thermally processed products identified the following critical factors that influence the level of contamination of the final products:

- reducing sugars, asparagine and other free amino acids contents in the starting raw material
- temperature profile applied during processing/production
- pH
- water content
- use of additives in the given recipe.

Reduction of acrylamide content can be achieved by modifying the processing conditions or choosing less risky cooking procedures, but the selection of suitable raw material is not simple, because

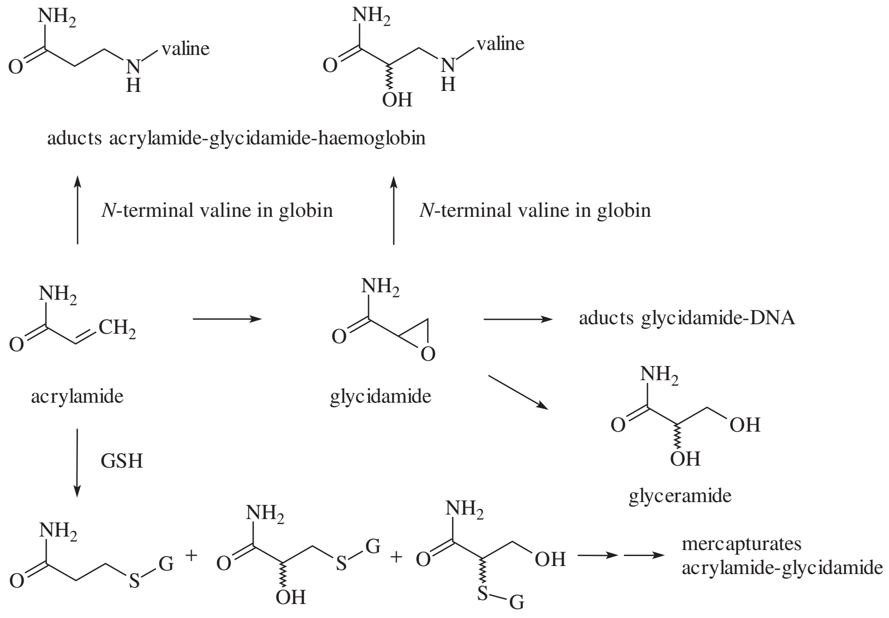


Figure 12.4 Metabolism of acrylamide in organism (GSH = reduced glutathione).

the levels of acrylamide precursors in crops are dependent on the particular cultivar, pedological and climatic conditions in a given locality and on the conditions used for storage of the raw materials.

Asparagine is an amino acid essential for plant growth, and reducing sugars are natural products of plant metabolism, but their concentrations are highly variable. While the molar ratio of sugars to asparagine in potatoes is relatively low, in cereals, which are materials with relatively high carbohydrate contents, free asparagine is a limiting component. Storage conditions do not significantly affect the asparagine content, but in potatoes temperature plays an important role. It is known that long-term storage of potatoes below about 4 °C increases the level of reducing sugars (see Section 4.2.2.6).

The strategy applied to reduce the formation of acrylamide during thermal operations is based on knowledge of the Maillard reaction mechanisms. For instance, the fermentation processes in the production of bread and pastry, leading to a decrease of dough pH, reduce the acrylamide formation during baking (the effect is based on protonation of asparagine amino group, which inhibits the reaction with carbonyl groups of sugars), but at the same time can intensify the formation of another technological contaminant, 3-chloropropane-1,2-diol (3-CPD, see Section 12.2.4). Table 12.2 illustrates the complexity of the optimal strategy suitable for reducing levels of technological contaminants.

Knowledge of acrylamide precursors also led to an urgent recommendation not to use rising agents based on ammonium carbonate in the production of bakery products, as the products (such as gingerbread) may contain high concentrations of this contaminant.

Table 12.2 Effect of dough acidity on the contents of acrylamide and 3-chloropropane-1,2-diol (3-CPD) in backery products.

Dough pH	Acrylamide (μg/kg)	3-Chloropropane-1,2-diol μg/kg)
3.1	93	40
4.0	105	27
5.0	125	19
6.0	145	14
7.0	163	11
8.0	187	9

Important information was also obtained from kinetic studies on acrylamide formation. When frying potato chips, the acrylamide formation correlates with temperature, its duration and colour of products (Table 12.3).

The changes in the amount of acrylamide produced during the course of the roasting of coffee is quite different. After a sharp increase in its content to about 12 500 μg/kg in about 4–5 min of roasting at 230 °C, a gradual decrease in acrylamide content follows, and after 25 min of roasting the acrylamide content is 6000 μg/kg, and after 50 min only 2000 μg/kg, which could be partly due to acrylamide losses caused by its volatilisation, but the main losses may be attributed to acrylamide reactions with components of roasted coffee beans. The decrease of acrylamide content continues during coffee storage and in ground roasted coffee, and results in a loss of 40% of acrylamide during 6 months of storage.

Many original solutions have been designed to reduce the formation of acrylamide. For example, the fortification of raw materials with common amino acids, such as glycine, reduces the overall yield of acrylamide, due to glycine competition in the Maillard reaction. Practical applications, especially in the production of biscuits and other bakery products, have also been found for commercial preparations of asparaginase, an enzyme that effectively breaks down the key precursor of acrylamide asparagine. An important criterion for assessing the effectiveness of measures to increase the safety of the product is an assessment of their impact on the sensory quality of products. The extensive scientific knowledge on the formation of acrylamide has resulted in a number of recommendations for food producers and for consumers, the most important of which are summarised, as an example, in Table 12.4. Compliance with these recommendations can contribute to a reduced dietary intake of acrylamide.

Under certain circumstances, acrylamide may infiltrate into drinking water treated using polymers based on acrylamide, which are used as flocculants. With regard to the toxicity of acrylamide and health risks associated with its intake, the hygienic limit for water intended for human consumption is 0.1 μg/l (European Council Directive 98/83/EC).

12.2.3 Furan

12.2.3.1 Structure and nomenclature

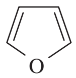
Furan is a simple heterocyclic compound (**12-20**), which typically arises by thermal decomposition of natural materials containing

Table 12.3 Changes of acrylamide concentrations in potato chips, depending on the temperature and time of frying.

Temperature (°C)	Time (min)	Acrylamide (μg/kg)	Temperature (°C)	Time (min)	Acrylamide (μg/kg)
130	7.0	12 000	170	2.3	26 000
140	6.0	14 000	180	2.1	32 000
150	4.7	18 000	190	1.6	38 000
160	3.0	22 000	200	1.3	47 000

Table 12.4 Measures to minimise acrylamide levels in different foods.

Food	Growing	Recipe	Processing
Potato chips	Selection of varieties with a low content of reducing sugars; storage at temperature >6 °C; control of reducing sugars contents		Thicker fries; blanching of raw fries in hot water to remove reducing sugars; control of temperature and frying time; frying at <170 °C; during frying of smaller batches reduce frying time; frying to a lighter surface colour
Bread	Important is sulfur fertilisation	No adding of reducing sugars; addition of calcium salts (carbonate, sulfate) can help	Control of temperature and cooking time; bake to a lighter crust colour



12-20, furan

cellulose and pentoses. It is a starting material for a number of industrial organic syntheses. Hydrogenation of furan, for example, yields tetrahydrofuran, which is used as a solvent.

The presence of this toxic substance in foods was demonstrated in the 1970s in studies of volatile compounds produced during the cooking of food. With regard to the low boiling point of furan (34.1 °C), its concentrations were not often quantified, as attention was mainly focused on the flavour and biologically active derivatives of furan formed mainly in the Maillard reaction. In this context the results presented in 2004 by the U.S. Food and Drug Administration (FDA), which documented relatively high levels of furan in various heat-processed food products, especially in roasted, pasteurised and sterilised foods, were very surprising. Information on the occurrence of this potentially carcinogenic technological contaminant inevitably led to a number of related studies and in the European Union, at the request of the European Food Safety Authority (EFSA), a working group was established by the Scientific Committee on Contaminants in the food chain (CONTAM), which was designed to gather information on this contaminant.

12.2.3.2 Occurrence, main sources and dietary intake

The highest furan concentrations were found in roasted coffee, caramel, canned baby foods (mainly vegetable foods) and many other canned foods. Examples of typical amounts of furan in a variety of foods are presented in Table 12.5.

The average daily exposure in babies is estimated to be 0.9 μg/kg body weight, in children up to 2 years of age it is 0.41 μg/kg body weight and in older children and adults it is 0.26 μg/kg body weight. The contributions of different foods to the total exposure of furan for adults are shown in Table 12.6.

12.2.3.3 Formation

The presence of furan has been demonstrated in many foods of very different composition, which shows that there are multiple possibilities for its formation. It has been found that the precursors of furan in foods may be various food components, including amino acids, carbohydrates and ascorbic acid, as well as unsaturated fatty acids and carotenoids.

12.2.3.3.1 Amino acids

The most important precursors of furan are amino acids that yield acetaldehyde and glycolaldehyde on degradation. Acetaldehyde is produced in the Strecker degradation of alanine (alanine is also the product of aspartic acid decarboxylation) or by decarboxylation and deamination of serine (see Section 2.5.1.3.2); glycolaldehyde results from the Strecker degradation of serine. Furan arises from these reactive intermediates by aldol condensation via 2-deoxyaldotetrose, which undergoes cyclisation and dehydration (Figure 12.5). Glycolaldehyde is additionally produced by fragmentation of reducing sugars.

12.2.3.3.2 Lipids

Furan precursors are oxidation products of polyunsaturated fatty acids, in particular of α-linolenic acid and linoleic acid. In the case of linolenic acid, the C–H bond cleavage at C-17 of (9*Z*,12*Z*,15*Z*)-17-hydroperoxyoctadeca-9,12,15-trienoic acid yields (9*Z*,12*Z*,15*Z*)-17-oxoheptadeca-9,12,15-trienoic acid. Subsequent autoxidation (primarily cleaved is the C–H bond at C-11 and the free radical isomerises) produces (9*Z*,11*E*,15*Z*)-13-hydroperoxy-17-oxoheptadeca-9,11,15-trienoic acid, which subsequently decays to form (*E*)-but-2-enal, which can isomerise to (*Z*)-but-2-enal (crotonaldehyde, Figure 12.6). In model experiments, the amount of furan increased by several orders of magnitude in the presence of iron ions.

Autoxidation of linoleic acid gives rise to (10*E*,12*Z*)-9-hydroperoxyoctadeca-10,12-dienoic acid as one of the major products, which is degraded to give (2*E*,4*Z*)-deca-2,4-dienal.

Table 12.5 Typical levels of furan in foods in the European market.

Foods	Furan (μg/kg)[a]		
	Mean value	Minimum	Maximum
Baby food			
Baby food with meat	4.0	3.0	6.0
Baby food with vegetables, including potatoes	40.3	4.0	153.0
Baby food with fruits	4.3	1.0	16.0
Fruit and vegetable juices	11.8	1.0	40.0
Vegetables			
Vegetables, canned	5.3	<2	12.0
Vegetables, fresh	<1	-	-
Fruits			
Fruits, canned	3.5	<1	6.0
Fruits, dry	2.6	<1	7.0
Soups			
Soups, canned	31.0	19.0	43.0
Meat and meat products			
Meat and meat products	3.0	<1	10.0
Meat, canned	9.0	4.0	14.0
Sauce mixtures, canned	10.1	<4	39.0
Bread			
Whole (white)	25.7	<4	148.0
Crust	65.6	5.0	193.0
Snacks and nuts			
Potato chips, peanuts, almonds	51.1	<5	143.0
Sweet biscuits	10.7	<2	24.0
Confectionery	10.3	4.0	20.0
Sugar	-	<1	<2
Caramel	484.4	17.0	1956
Pudding	-	<4	-
Soy sauce, acid protein hydrolysate	48.9	18.0	91.0
Coffee and coffee surrogates			
Coffee, ground	2677	959.0	5938
Coffee, brewed from grounds	78.0	13.0	199.0
Coffee, instant	929.0	44.0	2150
Coffee, brewed from instant	18.8	2.0	51.3
Chicory	282.0	282.0	282.0
Chicory beverage	10.5	6.0	15.0
Ice coffee, canned	106.0	106.0	106.0
Coffee beans, green	nd	nd	nd

Table 12.5 (*continued*)

Foods	Furan (μg/kg)[a]		
	Mean value	Minimum	Maximum
Coffee beans, roasted, stored in closed container	2129	1322	3027
Coffee beans, roasted, stored in unclosed container	856.5	22.0	1792
Milk and beverages			
Milko	nd	nd	nd
Chocolate	<2	<2	<2
Fruit juice	1.0	1.0	1.0
Coca-cola	<1	<1	<1
Beer (dark)	3.0	3.0	3.0

[a] nd = not determined (under limit of determination), < 'x' = under limit of detection for the given food.

Table 12.6 Dietary exposure to furan for adults from different foods.

Food	Estimated daily intake (μg/kg body weight)	Food	Estimated daily intake (μg/kg body weight)
Coffee (drink)	0.15	Cooked pasta	0.004
Cereals	0.01	Cooked beans	0.004
Salty snack	0.01	Sauces for pasta	0.001
Soups with meat	0.01	Juices	0.001
Pork and beans	0.004	Canned tuna	0.000 08

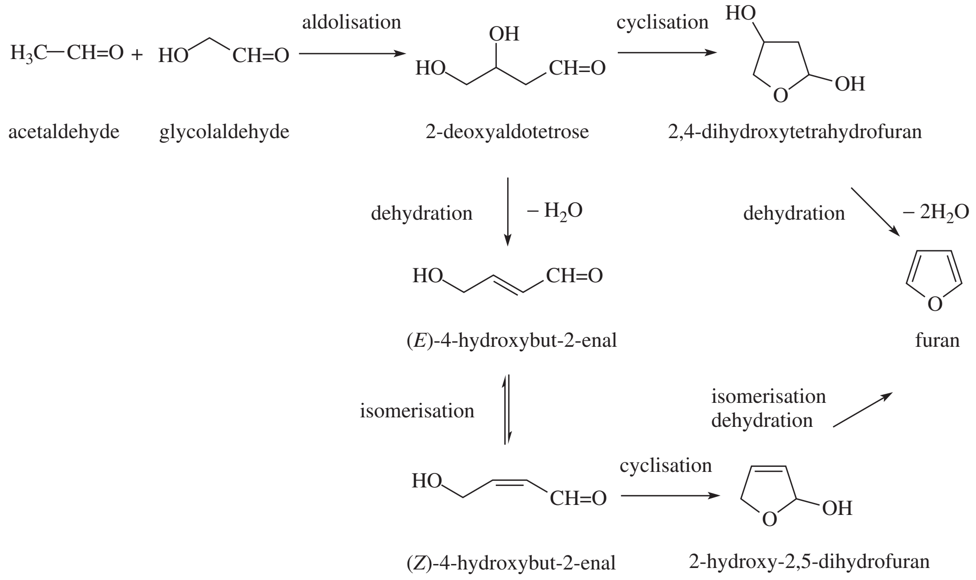


Figure 12.5 Formation of furan from amino acids.

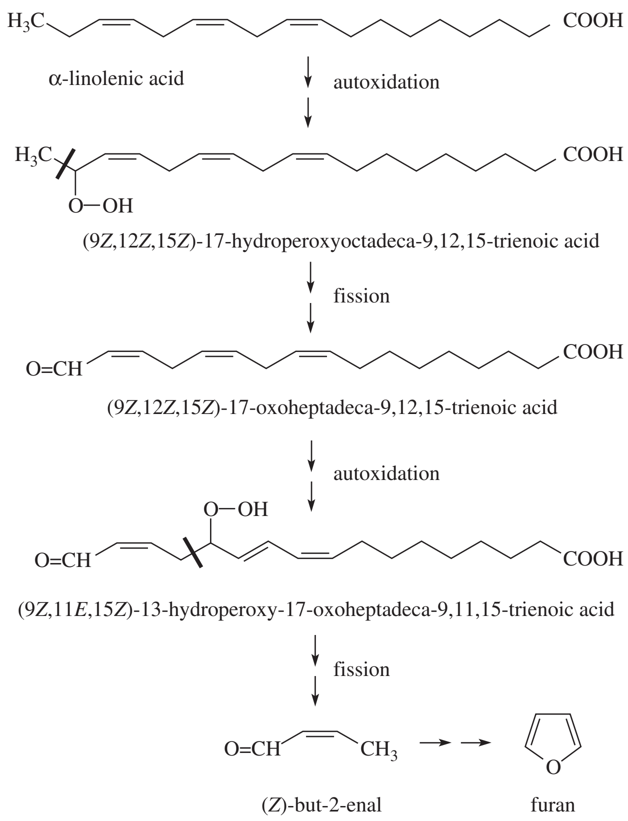


Figure 12.6 Formation of furan from linolenic acid.

Its isomer (2*E*,4*E*)-deca-2,4-dienal, formed by isomerisation, decomposes to (*E*)-but-2-enal and other products (Figure 12.7). A likely intermediate in polyunsaturated fatty acids, as well as in amino acids, is 4-hydroxybut-2-enal formed by oxidation of (*E*)-but-2-enal, which provides furan after cyclisation, isomerisation and subsequent dehydration.

12.2.3.3.3 Saccharides and related compounds

A Maillard reaction is another possibility for the formation of furan and its substituted derivatives. The highest amounts of furan were produced in model mixtures of alanine with glycolaldehyde heated to 250 °C. Serine in mixtures with ribose and sucrose gave 30%, and in mixtures with ribose or glucose there was only 10–25% formed. In the reaction mixture with erythrose there originated about an eight times higher amount of furan than in mixtures with glucose or fructose, as tetroses (their degradation products) are immediate precursors of furan. In terms of their potential to form furan, the following order of sugars can be compiled: D-erythrose > D-ribose > sucrose > D-glucose = D-fructose. The expected reaction mechanisms are summarised in Figure 12.8.

Four-carbon furan precursors are formed from hexoses in essentially three ways: via 1-deoxyhexo-2,3-diulose and aldotetrose, respectively), from 2-deoxytetros-3-ulose and 3-deoxyhexos-2-ulose. The most common 1-deoxyhexo-2,3-diulose is 1-deoxy-D-*erythro*-hexo-2,3-diulose, which is produced by 2,3-enolisation of ketosamines derived from glucose as the major intermediate and furan is also produced in a small amount from fructose in

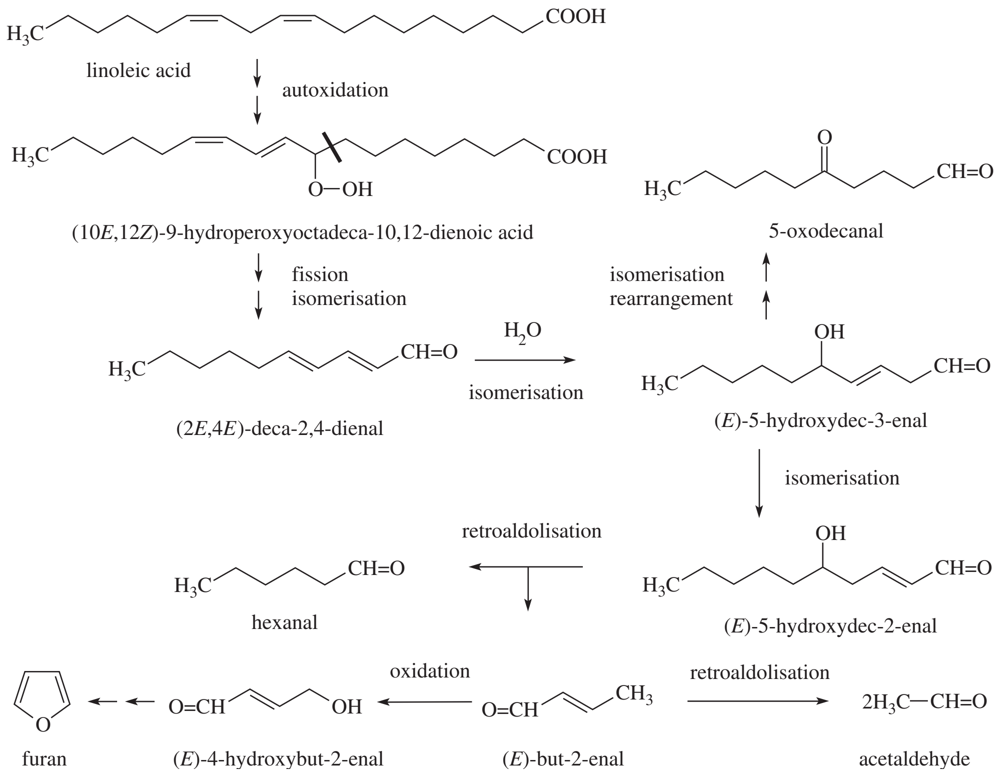


Figure 12.7 Formation of furan from linoleic acid.

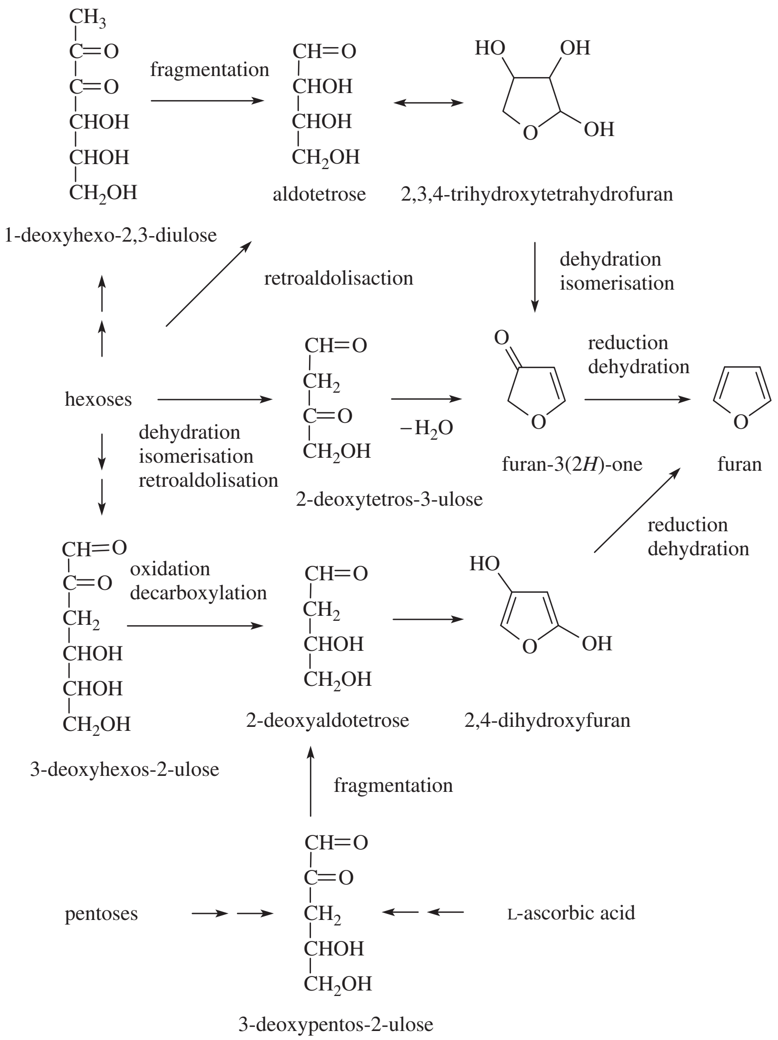


Figure 12.8 Formation of furan from saccharides and ascorbic acid.

the absence of amino compounds. Fragmentation of 1-deoxyhexo-2,3-diulose or hexose yields aldotetrose (carbons C-3, C-4, C-5 and C-6). Ribose gives rise to 1-deoxy-D-erythro-hexo-2,3-diulose, glucose or fructose. This pathway seems to be the most important pathway, since it provides approximately 50% of furan. Another precursor of furan, 2,3-deoxytetros-3-ulose, is produced as one of many products of the transformation (dehydration and retroaldolisation) of hexoses. The molecule of 2,3-deoxytetros-3-ulose contains the carbons C-1, C-2, C-3 and C-4 from the original skeleton of the hexoses. This pathway produces about 10% furan. From 2-deoxyaldotetrose, which is formed by oxidation, decarboxylation and cleavage of hexoses, carbon atoms C-2, C-3, C-4 and C-5 are incorporated into the furan molecule. This pathway produces about 10% furan.

Another natural furan precursor is L-ascorbic acid (the potential of dehydroascorbic and isoascorbic acids found in model experiments were still one order of magnitude higher). The reaction mechanism of furan formation from ascorbic acid is not yet fully understood. The expected precursor is 3-deoxypentos-2-ulose, which is in this case 3-deoxy-L-*glycero*-pentos-2-ulose, also known as 3-deoxy-L-xylosone (see Section 5.14.6.1.5).

12.2.3.4 Health and toxicological assessment

Furan is rapidly absorbed by the body due to its easy penetration across biological membranes. Its excretion from the body is also relatively fast. In the mammalian organism, furan is transformed under catalysis of cytochrome P450 complex. It seems that it is first oxidised (epoxidation of one double bond) and the oxidation product is then rearranged with opening of the cyclic structure. The reactive (*Z*)-but-2-ene-1,4-dialdehyde (**12-21**) formed is a strongly cytotoxic substance that can bind to proteins and nucleosides. An alternative metabolic pathway is its conjugation with glutathione. The resulting product is then expelled from the body through the urine.

12-21, (*Z*)-but-2-ene-1,4-dialdehyde

Based on toxicological studies in rodents, the IARC classified furan as a potential human carcinogen of Group 2B. The existing experimental data show the relationship between liver cancer and the exposure dose. As is apparent from the EFSA documents, it is necessary to collect additional data for the critical risk assessment of dietary furan and to clarify the toxicity of intermediates produced by furan biotransformation.

12.2.3.5 Mitigation

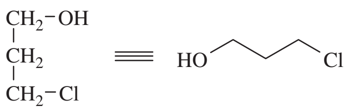
As mentioned above, the dietary sources of furan are mainly two groups of foods: cooked foods with relatively high water activity (products in cans or jars) and foods with low water activity processed by roasting or baking (such as coffee and bread).

The assumption that, after opening the cans or glass containers, furan quickly escapes into the atmosphere, due to its low boiling point, has proved to be untrue. During normal culinary procedures, furan has a relatively high retention in food, and a decrease of its concentration only occurs during intense boiling, when it is removed with the large volume of water vapour. The amounts of furan in different types of heat treatment have also been compared. Furan concentrations in industrially manufactured products were considerably higher than in home-made products, probably due to a diminished possibility of vapour release from the equipment. The research also showed that the furan content in industrially produced vegetable meals is significantly higher than that in fruit mixtures. When assessing these differences it is necessary to take into account the nature of the heat treatment and the content of furan precursors.

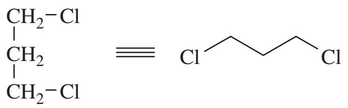
12.2.4 Chloropropanols and their esters

12.2.4.1 Structure and nomenclature

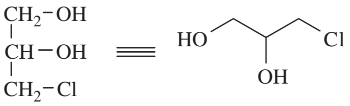
Chloropropanols (chlorinated propanols) are a group of three-carbon alcohols and diols with one or two chlorine atoms, which are hypothetically derived from glycerol. Six compounds, one chloropropanol (3-chloropropan-1-ol, **12-22**), two dichloropropanols (1,3-dichloropropan-2-ol, for short 1,3-DCP, **12-23** and 2,3-dichloropropan-1-ol, 2,3-DCP, **12-24**), two chloropropanediols (3-chloropropane-1,2-diol, 3-CPD, also known as 3-MCPD from 3-monochloropropane-1,2-diol, **12-25**, and 2-chloropropane-1,3-diol, 2-CPD or 2-MCPD, **12-26**) and structurally related 1,3-dichloropropane (**12-27**) arise as technological contaminants in acid protein hydrolysates (hydrolysed vegetable protein) and probably also in foods during the culinary and technological processing.



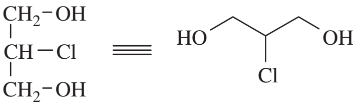
12-22, 3-chloropropan-1-ol (1-chloro-3-hydroxypropane)



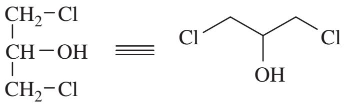
12-23, 1,3-dichloropropane (trimethylenedichloride)



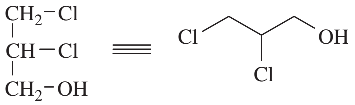
12-24, 3-chloropropane-1,2-diol (glycerol-1-chlorohydrine)



12-25, 2-chloropropane-1,3-diol (glycerol-2-chlorohydrine)



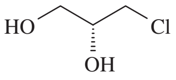
12-26, 1,3-dichloropropan-2-ol



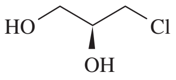
12-27, 2,3-dichloropropan-1-ol

1,3-DCP and its isomer 2,3-DCP were identified in hydrolysed vegetable protein in 1978, while the main chlorine-containing substance, 3-CPD, was found in 1982 and its 2-CPD isomer was identified in 1987 by Czech scientists. At the beginning of the 1990s, some CPD and DCP isomers were found in soy sauces, where hydrolysed vegetable proteins were used in the manufacture. A surprising finding was that these contaminants occur in a number of common foods where hydrolysed vegetable proteins are not used in the production.

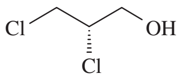
3-CPD and 2,3-DCP are chiral compounds, which occur in protein hydrolysates and foods as racemic mixtures of corresponding enantiomers (−)-(*R*)-3-chloropropane-1,2-diol (**12-28**), (+)-(*S*)-3-chloropropane-1,2-diol (**12-29**), (*R*)-2,3-dichloropropan-1-ol (**12-30**) and (*S*)-2,3-dichloropropan-1-ol (**12-31**), respectively.



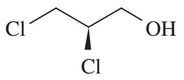
12-28, (*R*)-3-chloropropane-1,2-diol



12-29, (*S*)-3-chloropropane-1,2-diol

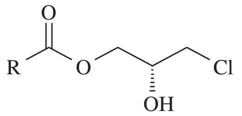


12-30, (*S*)-2,3-dichloropropan-1-ol

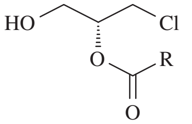


12-31, (*R*)-2,3-dichloropropan-1-ol

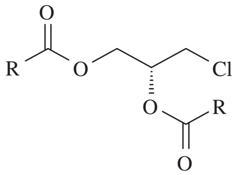
In 1980, it was found that hydrolysed vegetable proteins contain esters of chloropropanols with higher fatty acids. As examples, structures of esters derived from 3-CPD are shown in formulae **12-32** to **12-37**. Monoesters (alkanoic acid 2-chloro-2-alkanoyloxymethyl ethyl esters) and diesters (alkanoic acid 2-chloro-1-alkanoyloxymethyl ethyl esters) of 3-CPD were found in one sample of goats' milk in 1984, but it was understood that they were exogenous contaminants. The current research, focusing on DCP and CPD esters in foods, began in 2004.



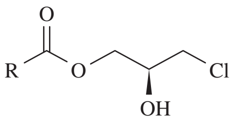
12-32, (*R*)-1-acyl-3-chloropropane-1,2-diol



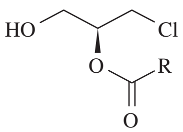
12-33, (*R*)-2-acyl-3-chloropropane-1,2-diol



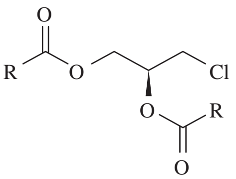
12-34, (*S*)-1,2-diacyl-3-chloropropane-1,2-diol



12-35, (*S*)-1-acyl-3-chloropropane-1,2-diol



12-36, (*S*)-2-acyl-3-chloropropane-1,2-diol



12-37, (*S*)-1,2-diacyl-3-chloropropane-1,2-diol

12.2.4.2 Occurrence, main sources and dietary intake

12.2.4.2.1 Protein hydrolysates

Protein hydrolysates used for flavouring dishes are basically divided into two large groups:

- chemical (acid) hydrolysates originating from Europe
- enzymatic hydrolysates originating from the Far East.

The first commercial acid protein hydrolysate, known as soup seasoning, was manufactured in Switzerland by J.M.J. Maggi in the early 1920s. Acid hydrolysed proteins are traditionally prepared by hydrolysis of vegetable materials rich in proteins (oilseed extraction meals, wheat gluten and other materials) with about 20% HCl, typically at temperatures exceeding 100 °C (at atmospheric or slightly elevated pressure) for about 8 h. Nitrogen contained in amino acids resulting from the hydrolysis of proteins represents 35–55% of the total nitrogen content. After cooling to 90–100 °C, the crude hydrolysate is neutralised with sodium carbonate or sodium hydroxide over 1.5–3 h so that the final pH is 4.5–7. The insoluble hydrolysate portion, known as humins, is then removed by filtration and the filtrate is allowed to stand for several weeks and, after maturation, is filtered again. The resulting product (30–40% dry matter, 2–3% total nitrogen, usually about 20% sodium chloride) is used directly as a seasoning and also for the production of soup cubes, bouillons, dehydrated soups and certain types of soy sauces.

The best-known enzymatic hydrolysate is soy sauce, which originated in China around 500 BC. The Chinese priest Zen reportedly introduced its production in Japan in 1250 AD. Japanese soy sauces known as honjōzō hōshiki are produced exclusively by fermentation, shinshiki hōshiki sauces contain 30–50% of enzymatic hydrolysate and 50–70% of acid hydrolysate, and aminosanekikongō hōshiki sauces are pure acid protein hydrolysates.

The major contaminant in acidic protein hydrolysates is 3-CPD. Other contaminants are mainly 2-CPD, 1,3-DCP and 2,3-DCP. Employing the above-described conventional technological processes of hydrolysate production, the ratio of these contaminants is roughly as follows: 3-CPD : 2-CPD : 1,3-DCP : 2,3-DCP = 1000 : 100 : 10 : 1. The resulting hydrolysates contained 100–800 mg/kg of 3-CPD, 10–90 mg/kg of 2-CPD, 0.1–6 mg/kg of 1,3-DCP and 0.01–0.5 mg/kg of 2,3-DCP. Their actual amounts depend primarily on the content of lipids in the raw proteinaceous material, the concentration and amount of hydrochloric acid and the temperature during hydrolysis. Chloropropanols are also found in soy sauces made from mixtures of acid and enzymatic protein hydrolysates. Soy sauces produced exclusively by traditional fermentation do not contain chloropropanols.

Since the 1980s, new technological processes have gradually been introduced as substitutes for conventional ones, which have led to a decrease of the chloropropanols content in acid protein hydrolysates and simultaneously in soy sauces. This trend is documented in the results of controls carried out by MAFF (Ministry

Table 12.7 Content of 3-CPD and 1,3-DCP esters in raw neutralised hydrolysate and humins.

Esters	Content (mg/kg)	
	Raw neutralised hydrolysate	Humins
3-CPD diesters	4	35
3-CPD monoesters	35	205
1,3-DCP esters	8	65

of Agriculture, Fisheries and Food) in the United Kingdom in 1990, when 23 out of 39 hydrolysates had a 3-CPD content higher than 10 mg/kg and 33 had greater than 1 mg/kg. In 1992, 17 out of 34 hydrolysates had a 3-CPD content lower than 1 mg/kg, and four hydrolysates analysed in 1998 had a content of 3-CPD within the limit of determination, to 0.428 mg/kg. Of the 50 hydrolysates analysed in 1999, 3-CPD was detected in 21 samples, nine of them contained amounts from 0.01 to 0.02 mg/kg, eight from 0.02 to 0.05 mg/kg, three from 0.05 to 0.1 mg/kg and concentrations higher than 0.1 mg/kg were found in nine samples. The highest amount of 3-CPD, however, was only 2 mg/kg. In the hydrolysates and soy sauces analysed in 2000, 3-CPD was found in amounts higher than 0.02 mg/kg in 25 cases, and in quantities of more than 1 mg/kg in 16 cases. The highest amount of 3-CPD was 82.8 mg/kg. 2-CPD was found at a level of 17.6 mg/kg in 26 samples. 1,3-DCP was detected in 17 cases (its concentration ranged from 0.006 to 0.345 mg/kg), 2,3-DCP was present in 11 samples and its amount ranged from 0.006 to 0.043 mg/kg. The same commodities analysed in 2002 contained 0.02 mg/kg of 3-CPD or higher in only seven cases, and the highest concentration was 35.9 mg/kg. A concentration of 1,3-DCP of 0.017 mg/kg was found only in the sample with the highest content of 3-CPD.

DCP and CPD esters of fatty acids (palmitic, stearic, oleic, linoleic and linolenic) were detected only in the crude neutralised hydrolysates (Table 12.7). These lipophilic compounds (the main fatty acid was oleic acid) in neutralised hydrolysates form a layer on the surface that is easily removed by filtration and goes to the waste humins, so that in the commercial products obtained from the crude hydrolysate they may be present only in traces.

12.2.4.2.2 Foods

Chloropropanols

Recent findings indicate that chlorinated propanols, especially 3-CPD and its positional isomer 2-CPD, are present in a number of common foods. Their amount in foods is generally lower than in acid protein hydrolysates. CPD isomers are present mainly in foods with lower water content, which have undergone industrial and culinary processing at higher temperatures (such as bread crust, toasted bread, roasted malt, barley and coffee). In other foods, such as processed and grilled cheese, meat products (such as fermented sausages) and fish, CPD isomers are present in smaller amounts. Relatively higher levels of these esters are present in smoked products (Table 12.8).

CPD may not always be simply an endogenous contaminant: for example, in smoked products it can be a component of liquid smoke preparations, which contain 200–760 μg/kg of CPD. It may also originate from acid protein hydrolysates used as condiments. High concentrations of 3-CPD (up to 1150 μg/kg) were, for example, found in chicken steaks. CPD can also migrate from some types of

Table 12.8 Content of 3-CPD in selected foods and other products.

Food	3-CPD (μg/kg)	Food	3-CPD (μg/kg)
Cereal products		***Meat products***	
Bread (white)	<10–55	Ground beef	<5
Bread crust	24–275	Regular patty (hamburger)	<10–71
Toasts	20–679	Ham (cured)	<5–22
Donats	11–24	Ham (smoked)	<10–47
Potato products		Salami	<10–69
Potato chips	<10–15	Sausages	<5–69
Potato crisps	15	Meat extracts	<10–14
Fish		***Various***	
Fish (baked)	<5–83	Coffee	<9–19
Fish (smoked)	<10–191	Malt	<10–850
Cheese	<10–95	Modified starches	<10–488
Fats and oils	<5–12	Garlic (thermally processed)	5–690

packaging that have been manufactured using chloromethyloxirane (also known as epichlorohydrin or 1-chloro-2,3-epoxypropane). In liquid smoke and garlic, CPD arises from unusual precursors. In the cellulose pyrolysates, acetol (3-hydroxyacetone) acts as a precursor, and in garlic CPD arises from allyl alcohol, which is a degradation product of alliin.

The amounts of other chloropropanols (2-CPD, 1,3-DCP and 2,3-DCP) are lower than the 3-CPD content. Higher levels of these contaminants are found sporadically, for example the concentration of 1,3-DCP in a sample of ham was <3–21 mg/kg, in beef steak 70 μg/kg and in various sausages ranged between <3 and 69 mg/kg.

Esters of chloropropanols

Mono- and diesters of 3-CPD with fatty acids occurring in foods represent a new group of contaminants, because they may release free 3-CPD by both chemical and enzymatic hydrolysis during food processing and possibly also by the action of lipases *in vivo*. In most cases, the content of 3-CPD esters is much higher than the amount of free 3-CPD (Table 12.9). Esters of 2-CPD have not been studied, but it is possible that they are likewise present in foods.

The highest amounts of 3-CPD esters (2500 mg/kg and more) undoubtedly occur in refined palm and olive oils, which are obtained from the pericarp of fruits (the outer and often edible layer in the fleshy fruits). The main proportion of 3-CPD esters arises in the deodorisation step, which takes place at temperatures exceeding 200 °C (see Section 3.4.3.7). Lower concentrations of 3-CPD esters occur in refined vegetable oils obtained from oilseeds, and the lowest amount of 3-CPD esters is found in virgin vegetable oils. Palm oil or mixtures of vegetable oils with palm oil are also used for frying foods, such as potato chips, crisps, cheeses and meat. Products containing refined palm oil include, for example, substitutes for milk powder (creamers), whipped creams in sprays, various biscuits, sweet spreads, soup cubes (broth) and other products, including infant and follow-on formulae. For example, the maximum measured levels of 3-CPD esters in infant formulae were 4196 μg 3-CPD/kg fat content, which corresponds to a concentration of 156 μg 3-CPD/l in ready-to-drink milk. Infants then could have a 3-CPD exposure of 25 μg/kg body weight per day, which is 12.5 times the TDI. During the examination of samples of follow-on formulae, a maximum level of 8467 μg 3-CPD/kg fat content was found, corresponding to 250 μg 3-CPD/kg in ready-to-drink milk. This can lead to intakes of 20 times the TDI. In the case of adults, the

Table 12.9 Content of 3-CPD esters in selected foods and other products.

Food	3-CPD esters (μg/kg)[a]	Food	3-CPD esters (μg/kg)[a]
Meat products		***Potato products***	
Salami	1399–1760	Potato chips	230–6100
Milk		Potato crisps	400[b]
UHT (3.5% of fat)	<3[b]	***Refined vegetable oils and other fats***	
Condensed (9% of fat)	67[b]	Palm oil	2821[b]
Milk, dry (26% of fat)	405	Palm kernel oil	1168[b]
Breast milk	<11–76	Coconut oil	1556[b]
Cereal products		Olive oil	<300–2462
Bread	6–85	Seed oils (mean value)	<300–1234
Bread crust	547[b]	Fats after frying	11 206[b]
Toasts	86[b]	Margarines	500–1500
Cookies	200–1690	***Animal fats***	
Coffee, coffee surrogates and malts		Butter, lard, tallow	<10–140
Coffee (roasted)	210–390	***Various***	
Rye (roasted)	145[b]	Sweet spreads	2300–10 300
Chicory root (roasted)	957[b]	Almonds, roasted nuts	433–1370
Barley (roasted)	1184[b]	Broths	380–670
Malt (Pilsener)	5–11	Cream in spray	50–730
Malt (roasted)	463–650	Baby and infant formulae	<72–8470

[a]Expressed in μg/kg of 3-CPD.
[b]Mean value.

daily intake could be up to five times the TDI in cases of high daily consumption of vegetable oils and margarines, which may contain up to 7356 μg 3-CPD/kg fat content.

Virgin oils from seeds (such as rapeseed, soybean and sunflower oils), by contrast, contain 3-CPD esters only at the level of <100–337 mg/kg, and virgin olive oils contain relatively very low amounts of 3-CPD esters (<100 to <300 mg/kg). The content of 2-CPD esters in fats and oils represents about 20–60% of the 3-CPD amount (palm oils usually contain more than 1000 mg/kg and seed oils less than 150 mg/kg of 2-CPD esters). Oils with a high content of 3-CPD esters may rarely also contain 1,3-DCP esters (up to 11 mg/kg).

Esters of glycidol and related compounds

In addition to esters of chloropropanols, commercially refined palm oils with higher 3-CPD content, contain esters of glycidol, also known as 2-(hydroxymethyl)oxirane or 2,3-epoxypropan-1-ol. Their amount increases with temperature during deodorisations. For instance, deodorising of palm oil for 6 h at 210 °C gives rise to 2800 μg/kg of 3-CPD esters and 300 μg/kg of glycidyl esters, deodorising at 250 °C for the same period yields 3300 μg/kg of 3-CPD esters of 2900 mg/kg of glycidyl esters. Glycidyl esters may become precursors of CPD esters and free CPD, respectively. Generally, the amount of glycidyl esters in refined oils and possibly also in other foods ranges from <100 to 4100 μg/kg, in refined palm oils they may occur at a level of 300–10 000 μg/kg, in margarines their amount ranges from <150 to 5000 μg/kg and in the fat of infant formulae they occur at a level of <150–3000 μg/kg.

12.2.4.3 Formation

The precursor of chloropropanols in acid protein hydrolysates is hydrochloric acid, which is used to hydrolyse the proteinaceous materials. Other precursors are residual lipids of the raw material and glycerol arising by hydrolysis of lipids (acylglycerols) with hydrochloric acid. Wheat gluten, which is often used as a raw material, contains, for example, 0.5–3.0% residual lipids, of which 30–36% are neutral lipids, mainly triacylglycerols, and about 60% are phospholipids. Soybean meal contains 1.0–3.0% lipids (of which about 30% represent neutral lipids and 60% phospholipids). The main precursors of chloropropanols are triacylglycerols, followed by phospholipids and glycerol. The content of glycerol in acid protein hydrolysates ranges from 200 to 3000 mg/kg.

The precursors of chloropropanols in common foods are naturally present or intentionally added chlorides (sodium chloride) and lipids and in some products also glycerol, which arises, for example, in dough as a result of the activity of yeast lipases. The primary reaction products are CPD diesters, which are hydrolysed to CPD monoesters. Esters of DCP are formed from CPD esters by reaction with chlorides. Free chloropropanols are then formed by hydrolysis of esters.

Exogenous sources of chloropropanols may be packaging materials containing epichlorohydrin copolymers with polyamines or polyamides that are used for the manufacture of tea bags, coffee filters and absorbents of packaged meat juices. Other exogenous sources of chloropropanols may be starches modified with epichlorohydrin, copolymers of epichlorohydrin with dimethylamine (used during sugar refining as flocculants or for the immobilisation of glucose isomerase in manufacturing of fructose syrups); polyelectrolytes based on epichlorohydrin and polyamines, which are used as flocculating and coagulating agents in the production of drinking water, are only relevant in certain cases.

12.2.4.3.1 Formation from acylglycerols

In acidic solutions, triacylglycerols are gradually hydrolysed to diacylglycerols, the diacylglycerols to monoacylglycerols and the final product is glycerol (Figure 12.9). A simplified sequence of reactions that lead from triacylglycerols through partial glycerol esters and 3-CPD and DCP esters to free CPD and DCP is shown in Figure 12.10. The main reaction that leads to DCP and CPD esters and also to free chloropropanols in acid protein hydrolysates and foods is the formation of reactive intermediates (cyclic acyloxonium cations), preferably from diacylglycerols (acyloxonium ions A and B in Figure 12.10) and monoacylglycerols. In particular, higher amounts of partial glycerol esters (diacylglycerols and monoacylglycerols) occur in oils obtained from seed pericarps (palm and olive oils), which is due to higher activity of naturally occurring lipases. Ring opening of cyclic acyloxonium cations with chloride ions yields 3-CPD and 2-CPD diesters, which may split off fatty acid(s) and yield cyclic acyloxonium ions and the corresponding hydroxy derivatives, respectively. Acyloxonium ions can also be formed by the elimination of fatty acids from monoacylglycerols. Reaction with chloride ions then leads to 3-CPD (2-CPD) monoesters and 1,3-DCP and 2,3-DCP esters. In the absence or depletion of chloride ions, the pathway leading from diacylglycerols is believed to end at the stage of glycidol esters. The ratio of 3-CPD and 2-MCPD arising from acylglycerols in acid protein hydrolysates depends on steric and other effects (terminal ester group), and is approximately 10 : 1. During the refining of vegetable oils (and possibly in foods) 3-CPD diesters, 3-CPD monoesters, 2-CPD diesters, 2-CPD monoesters and glycidol esters are produced at the ratio of about 49 : 14 : 28 : 7 : 7.

Glycidol esters are found in particularly high amounts in refined palm oil and palm oil-based fractions. Palm oil contains relatively high amounts of diacylglycerols (ranging from 2.3 to 4% in freshly extracted oils and from 4.0 to 7.8% in commercial oils) and monoacylglycerols (0.4–0.5%), therefore these acylglycerols are supposed to be the main precursors of glycidol esters. The formation of glycidol esters proceeds at high temperatures by an intramolecular rearrangement, followed by elimination of a fatty acid molecule that can be initiated by abstraction of the hydroxyl group's proton by the vicinal carboxyl group. The generated acyloxonium intermediate can then rearrange through charge migration, resulting in the release of a fatty acid and the oxirane ring formation by nucleophilic reaction of the alkoxide group. Analogous reaction (with elimination of water) may proceed with monoacylglycerols (Figure 12.10). In addition to glycidol esters, diacylglycerols heated at temperatures exceeding 140 °C in refined palm oil yield the

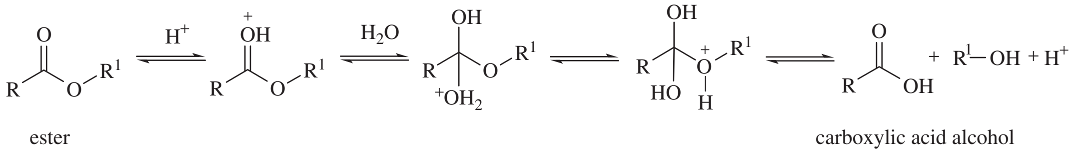


Figure 12.9 Mechanism of ester hydrolysis in acidic media.

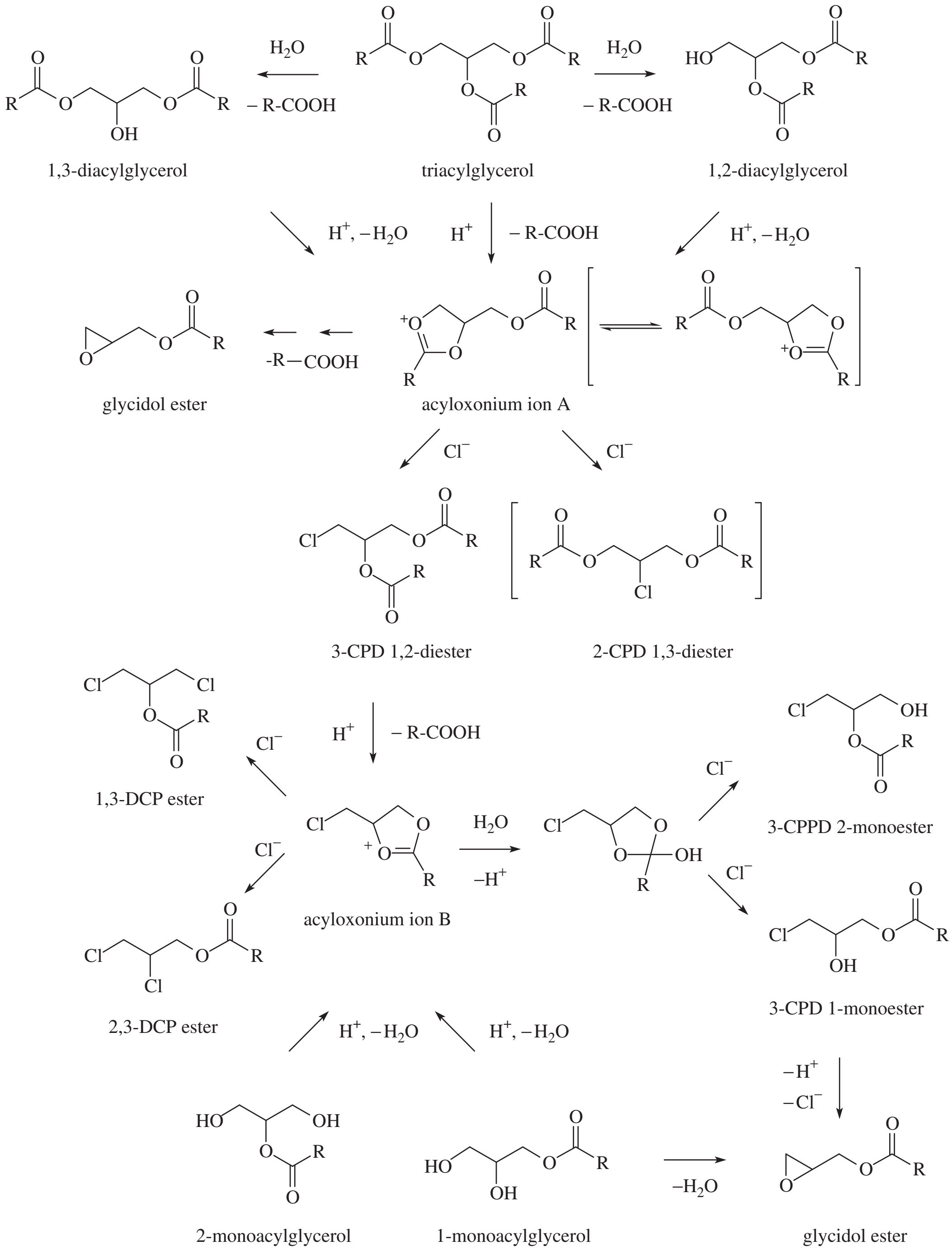


Figure 12.10 Formation of CPD and DCP esters from triacylglycerols and partial glycerol esters.

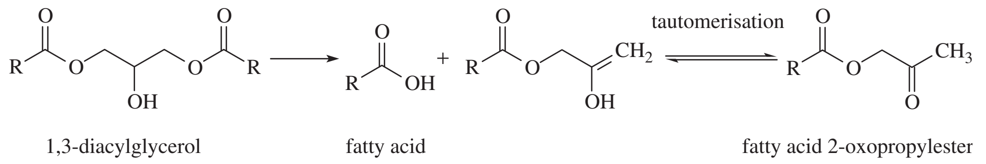


Figure 12.11 Formation of 2-oxopropyl fatty acid esters.

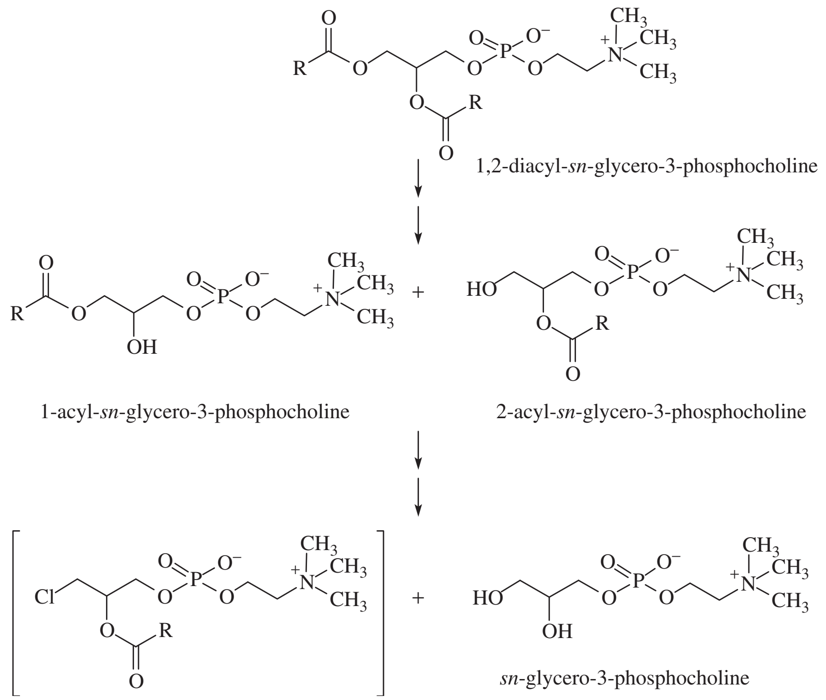


Figure 12.12 Formation of CPD from phospholipids.

corresponding oxopropyl esters at approximately 10% of glycidol esters levels (Figure 12.11).

12.2.4.3.2 Formation from phospholipids

Phospholipids are hydrolysed to deacylated products. For example, 1,2-diacyl-*sn*-glycero-3-phosphatidylcholine yields 1-acyl-*sn*-glycero-3-phosphatidylcholine, 2-acyl-*sn*-glycero-3-phosphatidylcholine and *sn*-glycero-3-phosphocholine. By seemingly analogous mechanisms to CPD esters formation (Figure 12.10), partial esters yield chloroester derivatives (such as 1-chloroester), which appears to hydrolyse to CPD esters and free CPD. CPD also arises from *sn*-glycero-3-phosphocholine (Figure 12.12). The ratio of 3-CPD and 2-CPD formed from phospholipids is about 4 : 1.

12.2.4.3.3 Formation from glycerol

Acid protein hydrolysates and many foods contain glycerol produced by hydrolysis of glycerolipids. Under acidic conditions, hydroxyl groups of glycerol can be protonated with the formation of alkyloxonium ions. In the case of the primary hydroxyl group, the next step is elimination of water, which is substituted by a chloride anion. This step is stereospecific and proceeds with inversion of the configuration at the carbon bearing the leaving group, which leads to a racemic mixture of 3-CPD. The alkyloxonium cation formed from the secondary hydroxyl group dissociates to a carbocation and water and the reaction of the carbocation with chloride ion yields 2-CPD (Figure 12.13). The ratio of 3-CPD and 2-CPD formed from glycerol is about 2 : 1. Elimination of water from glycerol simultaneously gives rise to hydroxyacetone and isomeric 2-deoxyglyceraldehyde.

Another possibility, which is envisaged in foods with low water contents during heat treatment at higher temperatures, is dehydration of the carbocation to a cation of glycidol, from which arises 3-CPD and 2-CPD by the opening of the oxirane ring by chloride ions. In this case the 3-CPD and 2-CPD ratio is about 3 : 1 (Figure 12.14).

12.2.4.3.4 Formation from allyl alcohol

In garlic and garlic-containing foods, 3-CPD and 2-CPD can result by the addition of hypochlorous acid (occurring, for example, in

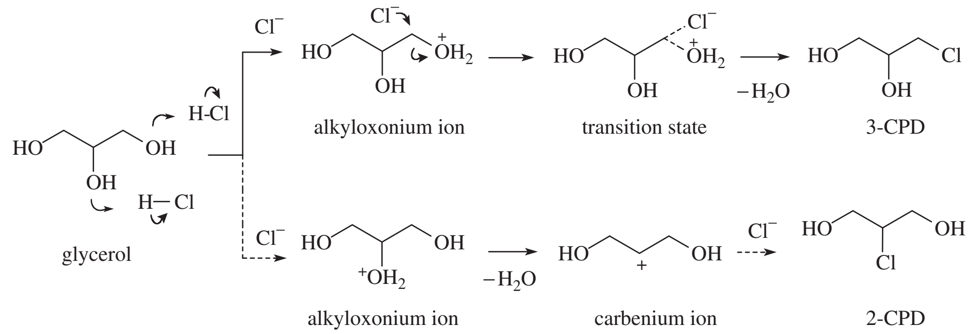


Figure 12.13 Formation of CPD from glycerol.

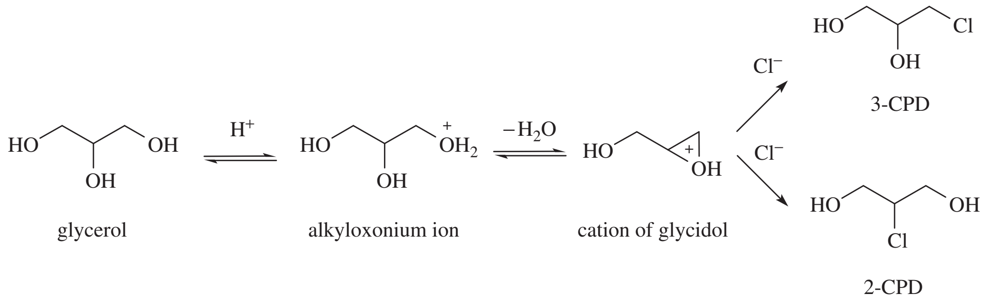


Figure 12.14 Formation of CPD from glycerol via glycidol.

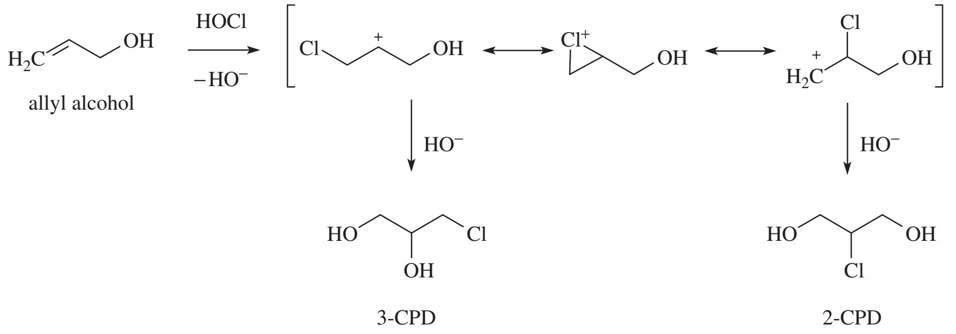


Figure 12.15 Formation of CPD from alliin.

chlorinated water) to allyl alcohol (Figure 12.15), which is one of decomposition products of alliin (Figure 8.7).

12.2.4.4 Health and toxicological assessment

Chloropropanols show various toxic effects. 3-CPD causes infertility in rats and suppression of the immune function and was shown to be genotoxic in several *in vitro* assays, but is not genotoxic and mutagenic *in vivo*. It was classified by the IARC as a chemical of the 2B group, and probably carcinogenic to humans.[3] In 2001, the Scientific Committee on Food (SCF) established a tolerable daily intake (TDI) for 3-CPD at 2 μg/kg body weight, and in 2002 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional maximal tolerable daily intake for 3-CPD, similarly at 2 μg/kg body weight. In the EU, maximum levels of 0.02 mg/kg for free 3-CPD in hydrolysed vegetable proteins and soy sauces were established in 2001. The maximum levels have been applied since April 2002 and are integrated into the Commission Regulation (EC) No. 1881/2006. These limits were not designed to account for 3-CPD esters. Data for 2-CPD and its esters are lacking and no toxicological data on esters of glycidol exist, but the degradation product glycidol and a metabolite 3-chlorolactic acid are genotoxic and carcinogenic compounds. In the human body the glycidyl esters are metabolised into free glycidol, a compound that is classified as possibly carcinogenic to humans (Group 2A compound) by IARC, as well as epichlorohydrin. 3-Chlorolactic acid, the main metabolite of 3-CPD in rats, was shown to be devoid of DNA-damaging effects *in vitro* in mammalian cells. 2-CPD is also a potential carcinogen.

The daily intake of 3-CPD by the adult population in the United Kingdom, for example, with an average consumption of foods with higher content of 3-CPD, is estimated at 0.10 μg/kg body weight, in the population aged 4–18 years at 0.18 μg/kg body weight and in children of up to 4 years at 0.28 μg/kg body weight. In populations consuming higher amounts of hydrolysates these estimates are higher (0.21, 0.38 and 0.58 μg.kg body weight, respectively). These values, of course, do not take into account the recent findings of high amounts of bound forms of 3-CPD (esters) in refined fats.

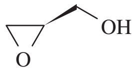
Genotoxic 1,3-DCP (a group 2B carcinogen)[3] also has other toxic effects (hepatotoxicity, nephrotoxicity and thyreotoxicity), which appear to be linked to its toxic metabolites and degradation products (epichlorohydrin and 1,3-dichloroacetone). Its positional isomer 2,3-DCP is also potentially genotoxic and carcinogenic. The concentration of 0.005 mg/kg 1,3-DCP in hydrolysates and soy sauces was recommended with respect to the current limit of quantification. The daily intake of 1,3-DCP by the adult population in the United Kingdom was estimated to be 0.051 μg/kg body weight and in children at 0.136 μg/kg body weight.

12.2.4.5 Mitigation

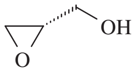
At the beginning of the 1980s, manufacturers of acidic protein hydrolysates started to implement practices that led to the reduction in the amount of chloropropanols. The stripping of crude hydrolysate with steam led to the removal of volatile chloropropanols, including 1,3-DCP and 2,3-DCP. Shortly after identification of non-volatile chloropropanediols, 3-CPD and 2-CPD, this procedure had to be replaced by decontamination of crude hydrolysate in a slightly alkaline medium. Other decontamination methods were also considered, such as degradation of CPD by microorganisms and enzymes, CPD extraction with ethyl acetate, butan-1-ol, butan-2-ol or isobutanol and removal of residual solvent by steam stripping. Some manufacturers have stopped production of traditional acidic protein hydrolysates and replaced them with aromatised enzymatic hydrolysates.

Table 12.10 shows some examples of the detoxified hydrolysates. Today, decontamination is performed on the crude hydrolysate cooled to a temperature of 90–100 °C, which is alkalinised to pH 8–9 with sodium hydroxide, potassium, ammonium or sodium carbonates, and dehydrohalogenation is allowed to proceed for 1.5–3 h. After decontamination, the hydrolysate is acidified with hydrochloric acid to have the same pH as the hydrolysate produced by traditional technology. Table 12.10 correspondingly illustrates one of the problems that emerged in this context. 2-CPD is much more stable than 3-CPD, is dehydrohalogenated more slowly than 3-CPD and accumulates in detoxified hydrolysates. For its removal, decontamination must be carried out at higher pH, higher temperature or for longer. The organoleptic properties of decontaminated hydrolysate are also somewhat different from the traditional hydrolysate.

The mechanism of dehydrohalogenation of vicinal chlorohydrins (α-chloroalcohols) is shown in Figure 12.16. Reaction in alkaline solutions is very rapid and the corresponding alkoxide (alcoholate) forms as the primary product. Important reaction intermediates are oxiranes (1,2-epoxides). By opening of the oxirane ring, the corresponding vicinal diols appear as the final reaction products. In the case of 3-CPD and 2-CPD, the dehydrohalogenation intermediate is glycidol and the final product is glycerol. Enantiomers of 3-CPD are decomposed at the same rate, and (*R*)-CPD gives rise to (+)-(*R*)-glycidol (**12-38**), and (*S*)-CPD yields (−)-(*S*)-glycidol (**12-39**) by inversion of configuration, known as Walden inversion.



12-38, (*R*)-glycidol



12-39, (*S*)-glycidol

Another problem is that glycidol, which is classified as a Group 2B carcinogen, accumulates under certain conditions in hydrolysates, because the rate of decomposition of CPD isomers is higher than the rate of decomposition of glycidol. For example, the maximum concentration of glycidol (after 5 min of decontamination at 65 °C at pH 9) reaches about a third of the initial CPD concentration. Glycidol is a very reactive compound which reacts not only with water, but also with many other nucleophilic reagents (alcohols, thiols, amines and acids). Its reaction with

Table 12.10 Concentrations of 3-CPD and 2-CPD in decontaminated hydrolysates.

Starting concentrations of 3-CPD and 2-CPD (mg/kg)	Conditions			Final concentrations (mg/kg)	
	Temperature (°C)	pH	Time (h)	3-MCPD	2-MCPD
95.7/15.1	100	8.5	3	<0.01	0.025
209/37.7	95	9.2	2	<0.01	0.072
209/37.7	95	9.2	2.5	<0.01	<0.01
50/22.5	93	8.6	3.25	<0.01	0.068
207/32.7	93	8.3	4.0	<0.01	0.01
207/32.7	92	8.4	3.5	<0.01	0.028
209/37.7	90	8.5	3.5	<0.01	0.28

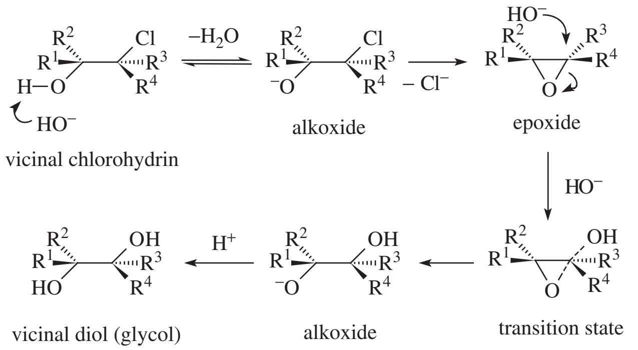
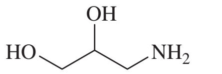
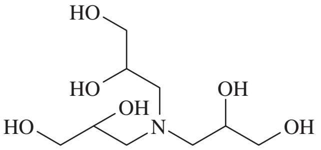


Figure 12.16 Dehydrohalogenation of vicinal chlorohydrins in alkaline media.

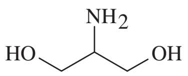
glycerol yields polyglycols and the main product with ammonia is 3-aminopropane-1,2-diol (**12-40**). Both enantiomers occur in acidic protein hydrolysates at a level of about 30 mg/kg. Another product is the tertiary amine tris(2,3-dihydroxypropyl) amine (2–4 mg/kg, **12-41**). With ammonia, 2-CPD yields 2-aminopropane-1,3-diol (serinol, **12-42**) as the main product. The reaction with the amino group of amino acids produces *N*-(2,3-dihydroxypropyl)amino acids (**12-43**). These reactions paradoxically contribute to the hydrolysate decontamination. Under alkaline conditions, L-amino acids isomerise to D-amino acids to some extent.



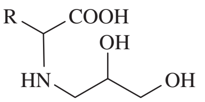
12-40, 3-aminopropane-1,2-diol



12-41, tris(2,3-dihydroxypropyl)amine

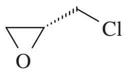


12-42, serinol

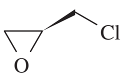


12-43, *N*-(2,3-dihydroxypropyl)amino acid

If 1,3-DCP is present in raw hydrolysates, under alkaline conditions both enantiomers of chloromethyloxirane, namely (−)-(*R*)-epichlorohydrin (**12-44**) and (+)-(*S*)-epichlorohydrin (**12-45**), are produced as decontamination products. (*R*)-2,3-DCP gives rise to (*S*)-epichlorohydrin, and (*S*)-2,3-DCP produces (*R*)-epichlorohydrin.



12-44, (*R*)-epichlorohydrin



12-45, (*S*)-epichlorohydrin

12.2.5 Polycyclic aromatic hydrocarbons

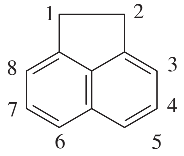
12.2.5.1 Structure and nomenclature

Polycyclic aromatic hydrocarbons (PAHs) and their derivatives represent an important group of virtually ubiquitous environmental contaminants, some members of which are characterised by their genotoxic and carcinogenic potential. Contamination of agricultural crops and food raw materials by exogenous PAHs is mainly due to polluted agricultural land or to air (atmospheric) pollution of the given locality. PAHs present in foods may also be endogenous contaminants, because considerable contamination of food can be caused by PAHs arising from natural precursors during some culinary or technological operations. For this reason, PAHs are classified as technological contaminants.

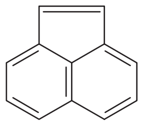
PAHs found in many foods, at least in trace amounts, contain 2–6 fused aromatic rings. In total more than 100 compounds have been identified. Compounds represented by formulae **12-46** to **12-71** are currently the most common PAHs, both in terms of their occurrence in the environmental components and in the context of health dietary risks for consumers. The symbol '*' indicates 15 priority PAHs according to the U.S. Environmental Protection Agency (EPA) and by the symbol '+' indictes 16 priority PAHs according to the European Scientific Committee on Food.[4]

Polycyclic systems, in which the adjacent rings have common atoms, may be *ortho*- or *ortho*-*peri*- or *peri*-condensed. If none of the common atoms are part of more than two rings, the system is *ortho*-condensed and can be either linear (such as in anthracene) or angular (e.g. in phenanthrene). The number of common atoms in this system is equal to twice the number of the common sides. The *peri*-condensed system exists if at least one of the common atoms belongs to three rings. The aggregate number of common atoms

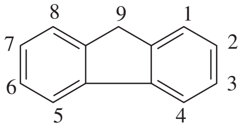
[4] In recent years, the evaluation of PAHs was carried out as part of the International Programme on Chemical Safety (IPCS), as well as by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and also by the EU Scientific Committee for Food (SCF). SCF was appointed by the European Commission in 1997 and in 2003 transferred to the European Food Safety Authority (EFSA). On the basis of available studies, the authority identified in 2002 a group of 15 priority PAHs, which can be regarded as potentially genotoxic and carcinogenic to humans. The basis for the recommendations were evidences of mutagenicity and genotoxicity for animal somatic cells *in vivo* obtained in different types of biological tests on experimental animals and conclusive evidence of carcinogenicity, except benzo[*ghi*]perylene, where data are insufficient. In 2005, JECFA confirmed the correctness of SFC recommendations and suggested to extend the priority list of PAHs by benzo[*c*]fluorene. When assessing the health risks in 2008, the EFSA Scientific Committee (CONTAM Panel) focused on food contaminants and also dealt with the question of the relevance of Toxicity Equivalency Factors (TEFs) for risk characterisation of PAH mixtures. This approach is commonly applied in assessing the contributions of individual components of mixtures of polychlorinated dibenzodioxins and dibenzofurans. Owing to the lack of data on the carcinogenic effects of individual priority PAHs in the case of oral exposure and taking into account the different mechanisms of their action, the formerly used TEF concept was rejected as scientifically unsupported (the prediction of carcinogenic effects of PAH mixtures based on TEF was not experimentally confirmed) and SCF suggested a system, which is based on knowledge of the effects of a marker of carcinogenic PAHs, benzo[*a*]pyrene and the data on the profiles of PAHs in foods evaluated on the basis of carcinogenicity studies of two characterised mixtures of coal tar in mice.



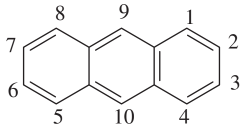
12-46, acenaphthene*



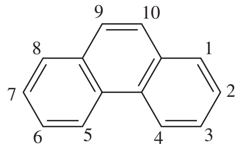
12-47, acenaphthylene*



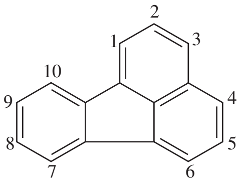
12-48, fluorene*



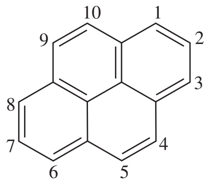
12-49, anthracene*



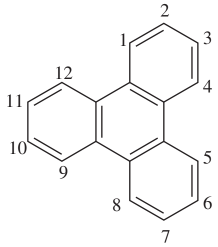
12-50, phenanthrene*



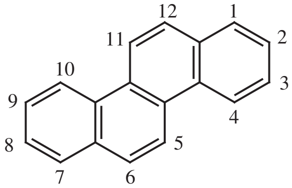
12-51, fluoranthene*



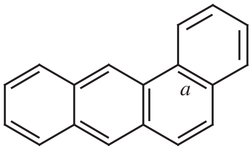
12-52, pyrene*



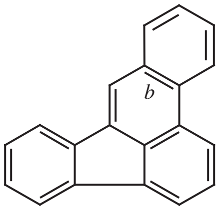
12-53, triphenylene



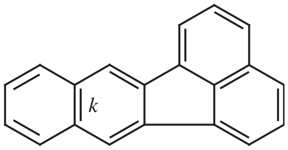
12-54, chrysene*+



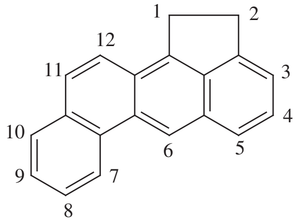
12-55, benzo[*a*]anthracene*+



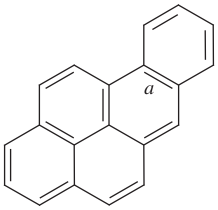
12-56, benzo[*b*]fluoranthene*+



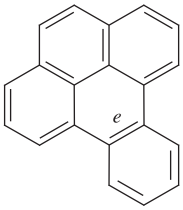
12-57, benzo[*k*]fluoranthene*+



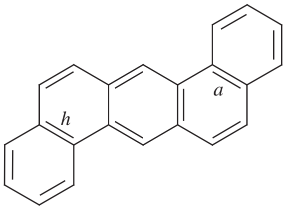
12-58, cholanthrene



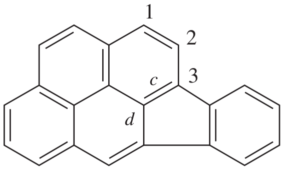
12-59, benzo[*a*]pyrene*+



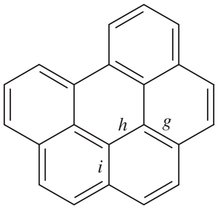
12-60, benzo[*e*]pyrene



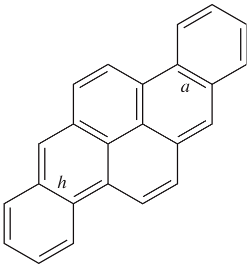
12-61, dibenzo[*ah*]anthracene*+



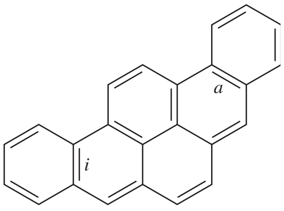
12-62, indeno[1,2,3-*cd*]pyrene*+



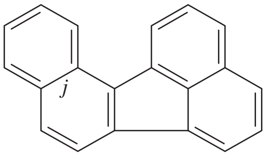
12-63, benzo[*ghi*]perylene*+



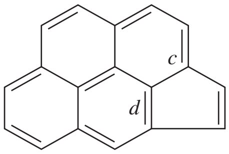
12-64, dibenzo[*ah*]pyrene+



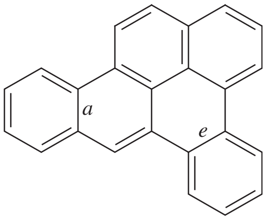
12-65, dibenzo[*ai*]pyrene+



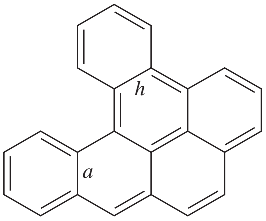
12-66, benzo[*j*]fluoranthene+



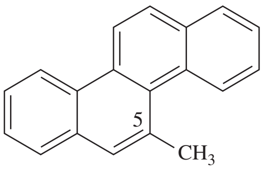
12-67, cyclopenta[*cd*]pyrene+



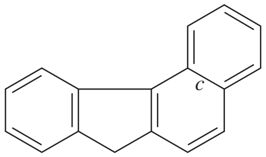
12-68, dibenzo[*ae*]pyrene+



12-69, dibenzo[*al*]pyrene+



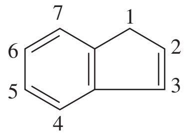
12-70, 5-methylchrysene+



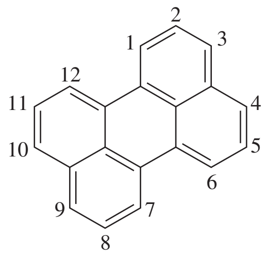
12-71, benzo [*c*] fluorene+

of several rings (at least three) is lower than twice the number of common sides. An example of such a hydrocarbon is pyrene.

For selected hydrocarbons, trivial names are used. Names of *ortho*- and *peri*-condensed polycyclic hydrocarbons that have no trivial names are formed from the name of a hydrocarbon, for which there is an appropriate trivial name, and a prefix indicating the nature and location of other rings, for example benzo[*a*]pyrene. The individual isomers are distinguished by letters *a*, *b*, *c*, etc., by which all peripheral sides of the basic hydrocarbon are identified, progressively from side *a* between C-1 and C-2. Examples of such isomers are benzo[*a*]pyrene and benzo[*e*]pyrene. The orientation of a formula should be such that the maximum benzene rings lie in a horizontal position, and also such that as many benzene rings as possible are lying at the top right of this horizontal axis of the molecule. The numbering of atoms is done in a clockwise direction, starting from the carbon atom of the ring of the far right and the furthest from the horizontal axis (carbons that cannot carry a substituent are not numbered). Besides the already mentioned hydrocarbons, the names of other contaminants are derived from indene (**12-72**) and perylene (**12-73**).



12-72, indene



12-73, perylene

Physico-chemical properties of PAHs, and correspondingly their fate in the environment and living organisms, are conditioned by the presence of conjugated double bond systems and the differences between individual hydrocarbons are determined by the number and relative position of condensed rings. PAHs are generally substances of low reactivity and the stability of linear condensed hydrocarbons generally decreases with the number of *ortho*-condensed rings. Angular and *peri*-condensed hydrocarbons are more stable. Melting points, boiling points, lipophilicity (measured by K_{OW}) and adsorption to soil or sediment particles (measured by K_{OC}) increase with an increase in the relative molecular weight of PAHs, but their vapour pressure (related to the value of Henry's law constant k_H) and water solubility decrease. The most important properties of selected PAHs are summarised in Table 12.11.

12.2.5.2 Occurrence and main sources

12.2.5.2.1 Main sources and penetration to environment

PAHs and their mixtures have been part of the human environment since time immemorial. They enter the ecosystem and the human food chain from anthropogenic sources (sources related directly or indirectly to human activities) and non-anthropogenic sources (natural, especially geochemical). However, the biosynthesis of these substances by some organisms has been demonstrated (Table 12.12).

Results of studies investigating the levels of PAHs in sediments or deeper layers of the polar icecaps show that, until approximately the end of the last century, a certain balance existed in the environment between production and degradation of these compounds. In other words, PAHs released by volcanic activity, fires and the existing human activities or biosynthesis, were eliminated from the environment by different degradation mechanisms to roughly the same extent, especially through photodegradation and microbial degradation. Current estimates of global annual emissions, however, refer to between tens of thousands and hundreds of thousands of tons of PAHs, which can contribute to a gradual increase in pollution of the atmosphere, terrestrial environment and hydrosphere. Up to 90% of this increase in PAH concentrations in environmental components is due to the burning of fossil fuels.

Transport in the environment occurs mainly through airflow and, in an aqueous environment, by re-suspension of contaminated sediments. These processes are associated with the solubility of PAHs in water (partition coefficient K_{OW}), their vapour pressure (Henry's law constant k_H), adsorption coefficient normalised to the organic carbon content of the soil or sediment (K_{OC}) and, last but not least, their chemical and biological stability. Some of these characteristics, especially k_H, K_{OW} and K_{OC}, correlate with relative molecular weights (M_r) of PAHs, and therefore PAHs are often divided into three groups:

- low molecular weight PAHs (referred to as light, $M_r = 152–178$ Da): acenaphthene, acenaphthylene, anthracene, phenanthrene and fluorine;
- medium molecular weight PAHs ($M_r = 202$ Da): fluoranthene and pyrene;
- high molecular weight PAHs (referred to as heavy, $M_r = 228–278$ Da): benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, benzo[*ghi*]perylene, chrysene, dibenzo[*ah*]anthracene, indeno[1,2,3-*cd*]pyrene, dibenzo[*ah*]pyrene and dibenzo[*ai*]pyrene.

12.2.5.2.2 Transport in the environment

A significant transport medium of a number of pollutants is air. PAHs are found there in the form of vapour or sorbed on solid aerosol particles. To date, almost 500 PAHs and their derivatives have been found in the atmosphere. It is reported that up to 75% of PAHs (especially the higher molecular weight compounds) in

Table 12.11 Selected physical and chemical properties of important PAHs.

PAH name	M_r (Da)[a]	Melting point (°C)	Boiling point (°C)	Solubility (mg/l)[b]	log K_{OW}[c]	k_H[d] (Pa m^3/mol)
Naphthtalene	128.2	80	218	30	3.37	43.01
Acenaphthene	154.2	96	279	3.47	3.92	12.17
Acenaphthylene	152.2	92	265–275	3.93	4.07	8.4
Fluorene	166.2	116	293	1.98	4.18	7.87
Phenanthrene	178.2	101	340	1.29	4.46	3.24
Anthracene	178.2	216	340	0.07	4.45	3.96
Fluoranthene	202.3	111	375	0.26	5.33	1.04
Pyrene	202.3	149	360	0.14	5.32	0.92
Benzo[*a*]anthracene	228.3	158	435	0.014	5.61	0.581
Chrysene	228.3	255	448	0.002	5.61	0.065
Benzo[*a*]pyrene	252.3	179	496	0.0038	6.04	0.046
Benzo[*b*]fluoranthene	252.3	167	481	0.0012	6.57	-
Benzo[*k*]fluoranthene	252.3	217	481	0.0055	6.84	0.016
Dibenzo[*ah*]anthracene	278.4	262	524	0.0005	5.97	-
Benzo[*ghi*]perylene	276.3	277	-	0.00026	7.23	0.075
Indeno[1,2,3-*cd*]pyrene	276.3	163	-	0.0062	7.66	-

[a] M_r = relative molecular weight.
[b] In water at 25 °C.
[c] Octanol-water partition coefficient.
[d] Henry's law constant.

Table 12.12 Main sources of PAHs in the environment.

Anthropogenic sources	Non-anthropogenic sources
Industrial	**Geochemical**
Production of heat and electricity	Coal
Coke production	Natural oil seepage
Production and processing of coal tar	Sedimentary rocks
Production, processing and use of asphalt	Hydrocarbon minerals (such as curtisite and idrialite)
Catalytic cracking	Volcanic activity
Machines with internal combustion	
Production and use of carbon black	
Wastewater, sewage sludge	
Food technology	
Non-industrial	***Biological***
Fires of forests and prairies, tanker crash	Biochemical syntheses by macrophytes and microorganisms
Open burning of waste	
Incinerators	
Tobacco smoking	
Household heating	

the atmosphere are bound to the surface of the respirable fraction of particles (particles with a diameter lower than 1 μm). More volatile PAHs (except fluoranthene) are associated primarily with larger particles (airborne dust, soot and others). The concentration of PAHs generally increases with a decrease in the atmospheric temperature. The total concentration of PAHs in air can be in the range of several orders. The background concentrations are in the range of 0.1–1 ng/m^3. In heavily polluted localities, the concentrations of PAHs may reach hundreds of ng/m^3 (expressed as the sum of 16 priority PAHs according to the U.S. Environmental Protection Agency, EPA). Mean concentrations in the air in urban and industrial areas are between 0.3 and 6 ng/m^3.

In air, PAHs are found sorbed to solid particles and as gases (aerosols). The dwell period of PAHs in the air is associated with a variety of processes, many of which also determine the extent of contamination of terrestrial vegetation, including crops. The level of dry and wet atmospheric depositions is significant. Particle-bound PAHs can be transported over long distances and are removed from the atmosphere through precipitation and dry deposition. PAHs are transported from surface waters by volatilisation and sorption to settling particles (sediments). Typically about 45% of the total PAH amount emitted is captured by vegetation, 10% is deposited in the soil, 5% in water areas and the remaining 40% is either chemically transformed or transported by air flow to more remote areas. In surface waters, PAHs are transformed by photooxidation, chemical oxidation and microbial metabolism. In soil and sediments, microbial metabolism is the major process for degradation of PAHs.

Data from southern England document the rising burden for soil by PAHs, arguably due to anthropogenic activities. The PAHs content in the surface soil layer (30 mm) has increased over the past 140 years from 18 to 130 μg/kg. The estimated half-life of soil retention is considerable, for example 371–387 days for chrysene, 229–309 days for benzo[*a*]pyrene and 361–420 days for dibenzo[*a,h*]anthracene. PAHs containing two or three benzene rings are volatilised more rapidly.

12.2.5.2.3 Occurrence in food raw materials and foods

The main source of contamination of fresh agricultural products and many other food raw materials is deposition of PAHs from air (vapours and dust particles), and to a lesser extent sorption from soil and water.

Contamination of vegetation

Knowledge on the mechanisms of exogenous contamination of terrestrial vegetation by PAHs is important both for assessing bioaccumulation, transformation and phytotoxicity (ecotoxicological aspects) and in terms of load monitoring of the human food chain (especially of agricultural crops) and then for the adoption of measures aimed at protecting human health. Plants receive PAHs by:

- absorption from water
- absorption through the root surface
- absorption through leaves from the vapour phase of air
- absorption through leaves from deposited particles.

The transmission of PAHs from soil to the plant root system is not very significant due to their high hydrophobicity. Its extent, however, depends on the water regime and the lipids content in the root cortex. For example, in carrots grown in soil with added sludge rich in PAHs (total content as the sum of 15 PAHs was 500 μg/kg), elevated levels were found, with 75% of the total content accumulated in the epidermal layer rich in lipids (200 μg/kg dry matter), while the content of PAHs in the edible part of the carrot was lower and was represented mainly by low molecular weight, more water-soluble PAHs (including fluorene, acenaphthene and phenanthrene). The intake of PAHs by leaves proceeds by direct intake through pores in the case of more volatile fractions, as well as through the cuticle from deposited dust particles containing sorbed contaminants. An important factor is the length of the growing season and characteristics of these aerial parts, which is why evergreen conifers are suitable indicators of environmental contamination. Results for PAHs in plants range from hundredths to tens of μg/kg. For example, the amounts of PAHs in different plant species were 22–88 g/kg in the leaves of trees, 48–66 g/kg in cereals, 0.05–50 g/kg in the above-ground parts of the vegetables, 0.01–6 g/kg in vegetable roots and 0.02–0.04 g/kg in fruits. In the vicinity of massive sources of contamination, however, the levels of PAHs in vegetation can reach 25 mg/kg. Knowledge of the rate of intake of PAHs by plants and their occurrence can be summarised as follows:

- low molecular weight PAHs are sorbed by plants more easily than hydrocarbons of higher molecular weights;
- one factor limiting intake of PAHs from contaminated soil is its composition, especially the concentration of carbon in organic compounds (with a higher content, PAHs are immobilised for potential transmission, due to strong sorption);
- PAH concentration in the above-ground parts of plants is higher than in roots;
- elevated PAH concentrations can be expected especially in plants with a high content of cuticular waxes and species with high leaf area and rough surface;
- PAH concentration is higher on the surface of plants than in the inner parts;
- vegetation and soil in the vicinity of emission sources are more contaminated than in more distant areas.

Contamination of animals

The source of PAHs to farm animals is mainly contaminated feed or water supply, but many animals are able to metabolise and eliminate these compounds, therefore their bioaccumulation is not as extensive as with persistent organochlorine compounds, such

as DDT and PCBs. For example, the value of the bioaccumulation factor (BAF) for benzo[*a*]pyrene in beef is about 0.003 mg/day.

Food chain uptake does not appear to be a major source of exposure to PAHs for aquatic organisms. The main source of PAHs is environmental contamination by oil products (indicators of oil substances in the aquatic ecosystem is fish). For example, areas with oil leakages (such as tanker accidents) may temporarily cause a significant increase in the levels of PAHs in fish muscle and especially in the gonads rich in fat, and the levels of PAHs in edible parts can be up to 500 times higher than the typical values. Normally, PAHs occurring in fish are gradually degraded and excreted, so the values of the bioconcentration factor are relatively low, but in crustaceans and other invertebrates, PAHs may significantly concentrate in tissues. Bioconcentration factors for fish are frequently in the 10–10 000 range, higher for compounds with higher relative molecular weight and lower for low molecular weight PAHs.

Occurrence in foods

A summary diagram of sources of PAHs in foods is shown in Figure 12.17. It is obvious that many food commodities can be contaminated from several sources. Data on the concentrations of PAHs in food have been obtained in a number of developed countries since the 1990s. Their comparison and generalisation, however, was quite difficult, not only due to differences in the spectrum of monitored compounds (the extent of contamination was often expressed as the sum of PAHs based on the priority list of EPA compounds), but also because different analytical methods were used and there was considerable uncertainty over the data obtained. Evaluation of the data available in 13 EU member states in 2004, however, documented the existence of increased contamination of certain food groups. Systematic monitoring implemented in EU Member States for the purposes of risk assessment was initiated by EFSA in 2005. It was recommended that 15 PAHs identified from a toxicological point of view were followed as a priority. The aggregated data are illustrated in Figure 12.18. Based on their evaluation, it was stated that benzo[*a*]pyrene, regarded for many years as a marker of food contamination by carcinogenic PAHs, does not fulfil this function optimally. Benzo[*a*]pyrene was found in at least 50% of the samples of a given commodity; however, in 30% of samples its concentration did not exceed the limit of detection, although the samples contained high concentrations of other PAHs with similar toxic potential. The facts illustrated by Figure 12.18 led scientists to consider the advisability of increasing the number of markers of contamination by carcinogenic PAH. With regard to typical levels of PAHs, three groups of PAHs were designed, representing 34%, 60% and 80% of the total amount of PAHs:

- PAH2: benzo[*a*]pyrene and chrysene
- PAH4: PAH2, benzo[*a*]anthracene and benzo[*b*]fluoranthene
- PAH8: PAH4, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, dibenzo[*ah*]anthracene and indeno[1,2,3-*cd*]pyrene.

The available data show that for an objective assessment of food contamination at least the PAH4 group (benzo[*a*]anthracene and benzo[*b*]fluoranthene) should be monitored, since 26% of samples contained at least one other carcinogenic PAH (very often chrysene). Figure 12.19 illustrates the average findings of the sum of eight PAHs in 33 food items, nine of which exceeded or closely approached 10 μg/kg. The highest amounts of PAHs found in tea (20% of samples actually exceeded the level of 100 μg/kg) cannot be directly associated with a significant exposure for consumers, because the infusion is very diluted.

Technological and culinary processes

Smoking Smoking is an age-old process of cooking and flavouring meat and fish over a low smoky fire, increasing their shelf life by partial dehydration and through the antibacterial properties of the absorbed smoke. Among the more than 300 products of

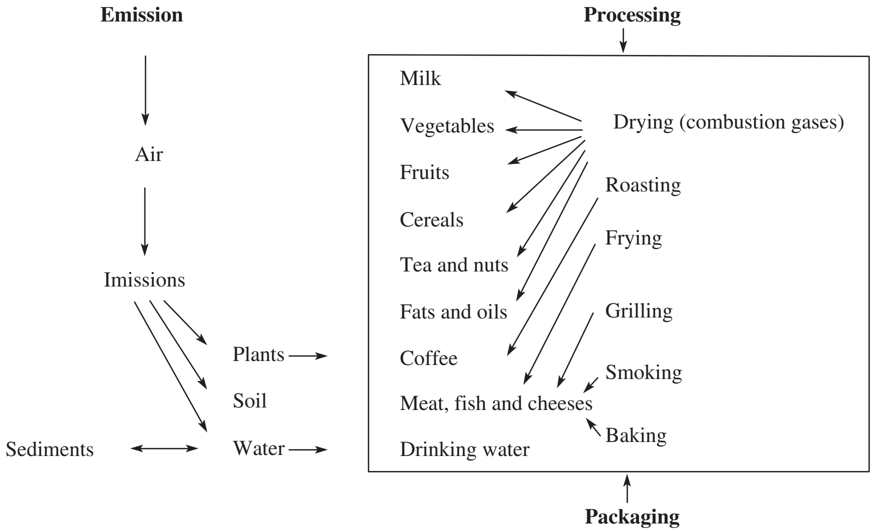


Figure 12.17 Possibilities of food contamination by PAH (emissions are measured directly at source, for example chimney, while imissions in the surrounding area).

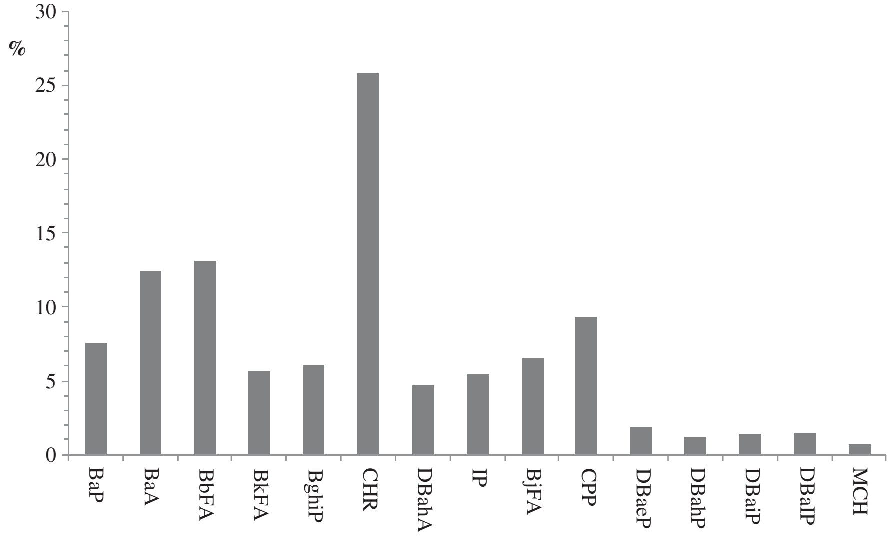


Figure 12.18 Relative average contribution of the EU priority PAHs to the overall food contamination (aggregated data for 1375 samples). BaP = benzo[a]pyrene, BaA = benzo[a]anthracene, BbFA = benzo[b]fluoranthene, BkFA = benzo[k]fluoranthene, BghiP = benzo[ghi]perylene, CHR = chrysene, DbahA = dibenzo[ah]anthracene, IP = indeno[1,2,3-cd]pyrene, BjFA = benzo[j]fluoranthene, CPP = cyklopenta[cd]pyrene, DbaeP = dibenzo[ae]pyrene, DbahP = dibenzo[ah]pyrene, DbaiP = dibenzo[ai]pyrene, DbalP = dibenzo[al]pyrene, MCH = 5-methylchrysene. According to Scientific Opinion of the Panel on Contaminants in the Food Chain on request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal 724, 1-114 (2008).

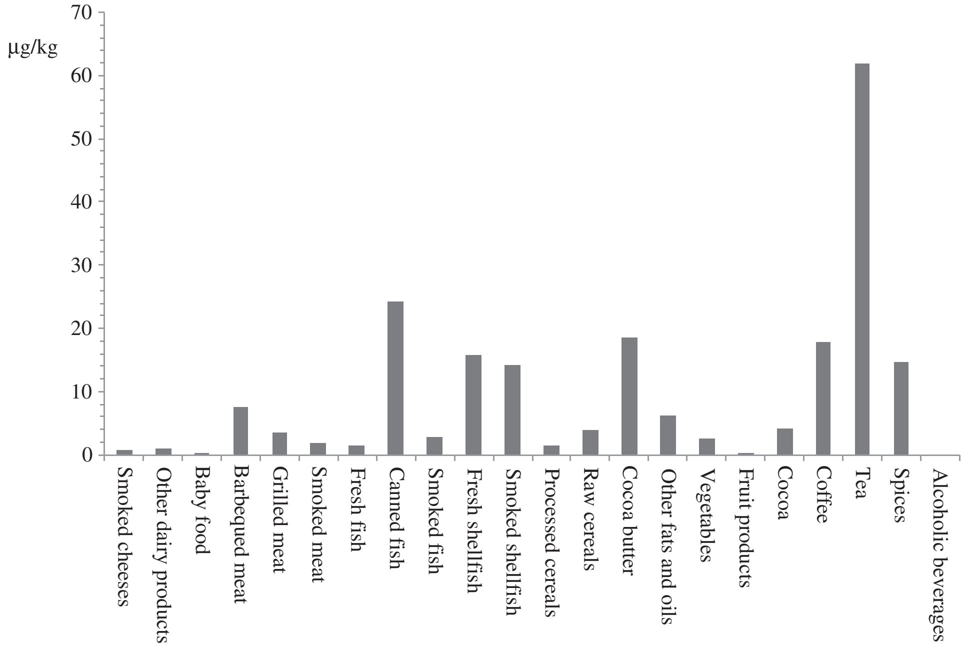


Figure 12.19 Mean values of PAH8 representing 33 food categories (agregated data for 4065 samples). According to Scientific Opinion of the Panel on Contaminants in the Food Chain on request from the European Commission on Polycyclic Aromatic Hydrocarbons in Food. The EFSA Journal 724, 1-114 (2008).

the pyrolysis of lignin, hemicelluloses, cellulose and other natural components of burnt wood or sawdust, about 70 PAHs have also been identified. Since carbon black dispersed in the released smoke contains the greatest proportion of PAHs (almost 90%), the major source of contamination is the surface of smoked foods. The resulting PAH content in the smoked product, however, depends on a number of factors. The critical factors are the fuel moisture, oxygen supply, technology of smoke formation, type of smoking

process (according to the temperature in the smoking room, it is classified as 'hot' or 'cold') and time of smoking. In practice hard wood is preferred (such as beech and oak wood), since it burns at lower temperatures and produces less carbon black compared with soft woods that contain a higher proportion of lignin, which is a major precursor of PAHs. Under normal conditions of smoke development at combustion temperatures in the range of 500–700 °C, smoke contains significant amounts of naphthalene, and the carcinogenic fraction of PAHs contributes to the total content of pollutants by about 5% wt. An illustration of the impact of smoking techniques on the content of benzo[*a*]pyrene in smoked products is shown in Table 12.13. In general, large differences are found between the products smoked in the home and in industrial smoke houses. Benzo[*a*]pyrene and benzo[*a*]anthracene concentrations in meat directly smoked over a fire can exceed tens of μg/kg, while concentrations of these substances in similar commercial products normally do not exceed 1 μg/kg. In modern food processing, the smoke evolution is automatically controlled and most PAHs (up to 90%) are removed from the smoke by filtration, washing and cooling.

A significant role in terms of content and distribution of PAHs is played by the type of smoked products, namely the size of surface exposed to smoke, and the products' fat content. The highest amount of PAHs is on the surface and their diffusion into deeper layers is not significant. For example, amounts of PAHs in the skin of smoked fish are between 4 and 24 times higher than in subsurface layers. A barrier against penetration of PAHs into the edible part may be formed by synthetic hydrophilic polymers, such as cellophane (regenerated cellulose). Currently, for flavouring meat, fish and other products such as cheese, smoking substances called liquid smoke are often used that have a controlled content of benzo[*a*]pyrene and other PAHs, which are below the acceptable limits.

Grilling Grilling or roasting on the grill (barbeque) is another form of heat treatment, temperatures of which vary over a wide range of from 150 to 400 °C. Grilling may, under certain circumstances, lead to very significant increases in both endogenous and exogenous PAHs in the product. Sources of contamination of its surface can be as follows:

- pyrolysis products of fat falling in drops onto the hot heater or into burning fuel;
- pyrolysis products of fuel used;
- pyrolysis products of grilled food generated endogenously in contact with the flame from food components.

A major impact of the grilling process on concentrations of benzo[*a*]pyrene in barbequed frankfurters is shown in Table 12.14. It is evident that from the hygienic–toxicological point of view it is especially risky to grill fatty materials in a direct flame, when the levels of contaminants may be up to several orders of magnitude higher than those of conventionally heat treated products.

Table 12.13 Influence of smoking conditions on benzo[*a*]pyrene concentration in smoked meat products of comparable parameters (surface, fat content and packaging).

Technology of smoke development (smoking technique)	Content of benzo[*a*]pyrene (μg/kg)
Smoldering smoke	<0.1–36
Moist smoke	0.1–0.8
Smoke from friction generator	0.1–4.6
Hot smoking (bright)	<0.1–2.1
Hot smoking (dark)	0.2–36
Cold smoking (light)	0.1–3.2
Cold smoking (dark)	0.1–56

Table 12.14 Content of benzo[*a*]pyrene in grilled frankfurters under various conditions.[a].

Heat source	Mean content (μg/kg)	Range of values (μg/kg)
Frying pan	0.1	Not detected–0.2
Electric oven	0.2	0.1–0.3
Charcoal	0.3	Not detected–1.0
Pine cones	18	2–31
Logs	54	6–212
Embers	8	<1–25

[a]Original content of 0.2 μg/kg.

Drying and roasting The amounts of benzo[*a*]pyrene in products dried by indirect heating are generally low, often below 0.1 μg/kg. Higher amounts, however, can be expected in the event of contact with combustion gases. A possible source of PAHs in the production of beer and whiskey is malt, which is also sometimes dried by direct heating. Considerable attention is paid to roasted coffee. Formation of phenanthrene, anthracene and benzo[*a*]anthracene in coffee beans was observed at temperatures above 220 °C, whereas formation of pyrene and chrysene required 260 °C. Low levels of benzo[*g*,*h*,*i*]perylene were also noted for dark roasting below 260 °C, with simultaneous partial degradation of three-ring PAHs, suggesting that transformation of low molecular PAHs to high molecular PAHs occurs as the degree of roasting is increased. The PAH transfer to the infusion was fairly moderate (<35%).

12.2.5.2.4 Exposure sources

Quite the most important source of human exposure to PAHs (typically >95%) is food (oral intake). Pollutants also penetrate the body by inhalation of contaminated atmospheric particles, which are the dominant source in smokers. Dermal exposure is generally

regarded as a rarity, with the exception of professionally exposed persons.

The first comprehensive studies focusing on the estimation of dietary exposure to individual PAHs were published in the 1990s; however, the comparability of these results was limited not only by differences in analytical methods and analytes (often it was the EPA priority list including the volatile fraction of PAHs), but also by differences in the food basket composition and calculation methodology. Table 12.15 summarises estimates of dietary exposure of EU consumers to benzo[*a*]pyrene and to PAH2, PAH4 and PAH8 groups of PAHs defined above in recent years. The EU database for average consumption of individual food categories was used for the calculation. Generally, the most important dietary sources of PAHs to the European population are cereals, cereal products and seafood. These categories were also used to calculate exposure to PAHs of extreme drinkers (Table 12.16). As a biomarker to assess exposures to PAHs of workers (e.g. in the petrochemical industry and in aluminium production plants) and also that of ichthyofauna and ruminants exposure, the major pyrene metabolite 1-hydroxypyrene, which is excreted into the bile, urine and milk, was employed.

Information on the occurrence of derivatives of PAHs in food is very limited, but their concentrations are lower than those of the parent PAHs. In recent years, considerable attention has been devoted to nitro derivatives of PAHs in view of their toxic potential. High amounts of 1-nitronaphthalene, 2-nitrofluorene and 1-nitropyrene were found in home-smoked meat, where their concentration reached tens of μg/kg. Surprisingly high contamination by nitro derivatives of PAHs has been demonstrated in some types of spices and tea. Typical concentrations generally do not exceed units of μg/kg.

12.2.5.3 Formation

Information on the mechanisms of formation of PAHs and derived substances are significant not only in terms of taking measures that minimise possibilities of their penetration into the food chain, but also to reduce endogenous contamination of food in some culinary or technological processes.

12.2.5.3.1 Formation of hydrocarbons

One of the most important routes of PAHs formation are combustion processes (pyrolysis) of organic matter with limited availability of oxygen at temperatures of 500–900 °C, and especially above 700 °C. The mechanism of PAHs formation is **pyrosynthesis**

Table 12.15 Exposure of consumers to benzo[*a*]pyrene, PAH2, PAH4 and PAH8 through selected foods.

Foods	Daily consumption (g)	Exposure (ng/day)			
		benzo[*a*]pyrene	PAH2	PAH4	PAH8
Cereals and cereal products	257	67	129	257	393
Sugar, sweets and chocolate	43	5	13	25	39
Fats (vegetable and animal)	38	26	112	177	239
Vegetables, nuts and legumes	194	50	124	221	378
Fruits	153	5	40	75	87
Coffee, tea and cocoa (drinks)	601	21	55	106	156
Alcoholic beverages	413	4	12	25	74
Meat and sausages	132	42	107	195	279
Seafood	27	36	140	289	421
Fish and fish products	41	21	84	170	210
Cheeses	42	6	12	20	30

Table 12.16 Daily dietary exposures to PAHs.

Diet type	Units	Benzo[*a*]pyrene	PAH2	PAH4	PAH8
Average	ng	235	641	1168	1729
High intake	ng	389	1077	2068	3078
Average	ng/kg body weight	3.9	10.7	19.5	28.8
High intake	ng/kg body weight	6.5	18.0	34.5	51.3

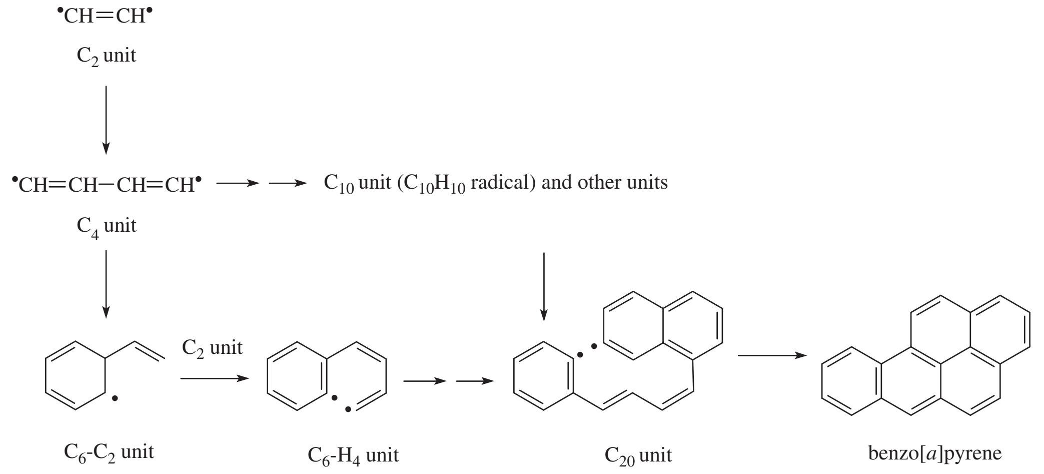


Figure 12.20 Formation of benzo[*a*]pyrene by pyrolysis of organic matter.

from low molecular weight unsaturated aliphatic hydrocarbons. Figure 12.20 illustrates the formation of benzo[*a*]pyrene by recombination of free hydrocarbon radicals. This pathway, for example, produces PAHs during combustion of fossil fuels. Flame temperature and oxygen availability determine their composition. Many fuels already contain some PAHs that can get into emissions directly. A range of natural compounds may be PAH precursors, such as plant steroids and other terpenoids. Figure 12.21 illustrates the main decomposition products of stigmasterol.

Another important pathway leading to PAHs is the thermal elimination reaction of benzene derivatives (Figure 12.22). For example, the elimination of water from phenolic compounds (X = OH) yields, via 1,2-didehydrobenzene (often incorrectly called benzyn), another reactive intermediate benzobicyclo[2,2,2]triene and naphthalene as a final product. The contribution of endogenously generated compounds to the total dietary intake of PAHs is not too significant as the contamination of food is largely exogenous.

12.2.5.3.2 Formation of hydrocarbon derivatives

In the evaluation of reactivity of PAHs, reactions that occur in the atmosphere are of particular interest, because the resulting products can contaminate crops. The extent and nature of these reactions depends on temperature, intensity of solar radiation and the concentration of substances, which are capable of electrophilic substitution on the aromatic nucleus. Reactions of PAHs with ozone, singlet oxygen, nitrogen or sulfur have been described, and particular attention was focused on reactions leading to PAH nitro derivatives, because of their high toxicity. Certain amounts of these contaminants are emitted directly into the atmosphere from sources formed during organic matter combustion, but they may also arise by radical reactions in the gas phase. PAHs (or amino-PAHs) generally react with free hydroxyl radicals ($HO^•$) to form reactive hydroxyl radicals of PAHs, which react with $^•NO_2$ radicals in the gas phase forming hydroxynitro derivatives of PAHs. Hydroxynitro derivatives eliminate water to form PAH nitro derivatives. This

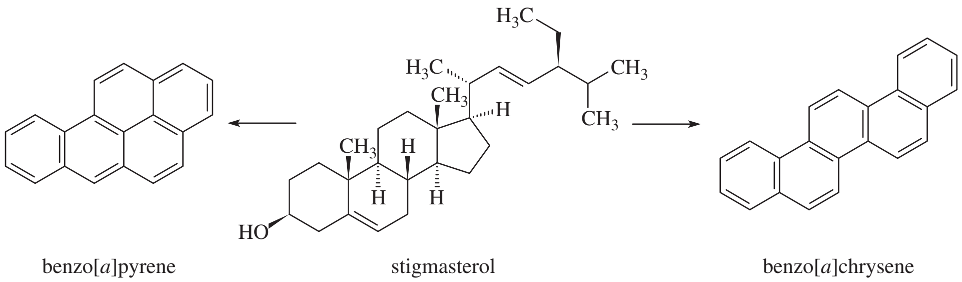


Figure 12.21 Formation of benzo[*a*]pyrene and benzo[*a*]chrysene by thermal decomposition of stigmasterol.

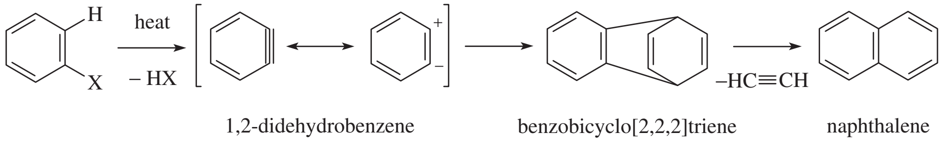


Figure 12.22 Formation of naphthalene from benzene derivatives.

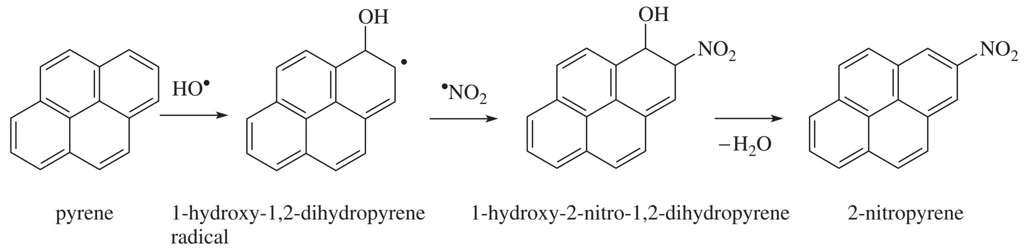


Figure 12.23 Formation of 2-nitropyrene in the atmosphere.

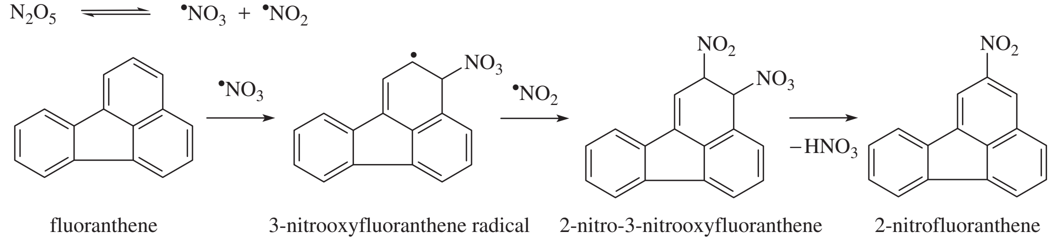


Figure 12.24 Formation of 2-nitrofluoranthene in the atmosphere.

reaction takes place in daylight. Another nitrating substance is nitric oxide (N_2O_5), which is cleaved to $^{\bullet}NO_3$ and $^{\bullet}NO_2$ radicals. The resulting radicals react with PAHs with elimination of nitric acid (HNO_2) yielding nitro-PAHs. Nitration of PAHs by nitric oxide radicals proceeds especially in the dark. Two different mechanisms of nitro-PAH formation can therefore be distinguished. Reactions of the first type that produce 2-nitropyrene in daylight are illustrated in Figure 12.23. The formation of 2-nitrofluoranthene in the absence of sunlight is illustrated in Figure 12.24. The amount of this compound produced is about the same as that generated during daylight.

12.2.5.4 Health and toxicological assessment

In the last decade a rigorous evaluation of the toxicity and health risks of PAHs associated with their long-term dietary intake has taken place. The acute toxicity of PAHs is relatively low; for mice and rats the LD_{50} of benzo[*a*]pyrene is >1600 mg/kg body weight. As indicated in Table 12.15, foods are clearly the most important source of PAHs (for non-smokers). To date, in terms of regulating polycyclic aromatic hydrocarbons (PAHs) in food, the EU has only established limits for benzo[*a*]pyrene, through European Commission regulation (EC) No. 1881/2006, which has hitherto been regarded as an indicator of the overall burden by PAHs. Maximum limits apply to baby foods and foods that contain mostly fats and oils, and for foods where smoking or drying processes might cause high levels of PAH contamination. For example, the maximum levels of benzo[*a*]pyrene in infant formulae and follow-on formulae, cereal-based and baby foods for infants and young children and dietary foods for special medical purposes for infants are 1 μg/kg, and for oils and fats (excluding cocoa butter), and the meat of fish other than smoked fish, the maximum levels were set at 2 μg/kg and to 5 μg/kg wet weight in smoked meats and smoked meat products. Maximum levels have likewise been set for fish and fishery products, where high levels of contamination may be caused by pollution of the environment (5 μg/kg for smoked fish, smoked fishery products, crustaceans and cephalopods and 10 μg/kg for bivalve molluscs). The EFSA recommendation to follow a sum of four PAHs, benzo[*a*]pyrene, chrysene, benzo[*a*]anthracene and benzo[*b*]fluoranthene has still not been incorporated into the legislation.

PAHs absorption in the gastrointestinal tract is generally easy; however its extent depends on food composition and varies over a range of about 12–99%. Low molecular weight PAHs are absorbed better. Absorption of PAHs increases with higher fat content, but some food components (such as flavonoids and other polyphenols) reduce the intake of PAHs. The bioavailability of PAHs from burnt food is surprisingly low, even though their content is relatively high, which is due to the absorption of PAHs by charred particles. Absorption of PAHs is followed by a rapid distribution in the body, since they can easily penetrate the lipoprotein membranes. With the exception of benzo[*a*]pyrene, the toxicokinetics of the priority PAHs are little documented. In general, however, thanks to extensive biotransformation, accumulation of PAHs in the human body (and correspondingly in other mammals) does not occur. Biotransformation of the individual PAHs is quite similar, but the quantity and rate of metabolite formation depend on the parent compound structure. In the initial stage of biotransformation, PAHs are oxidised by oxidoreductases of the cytochrome P450 complex, which are located in endoplasmic reticulum or microsomes of cells in various tissues (especially the liver) and are induced by PAHs or other inducers. So far, most attention in this respect has been devoted to benzo[*a*]pyrene, although some related compounds (nitro-PAHs or acridine derivatives) exhibit

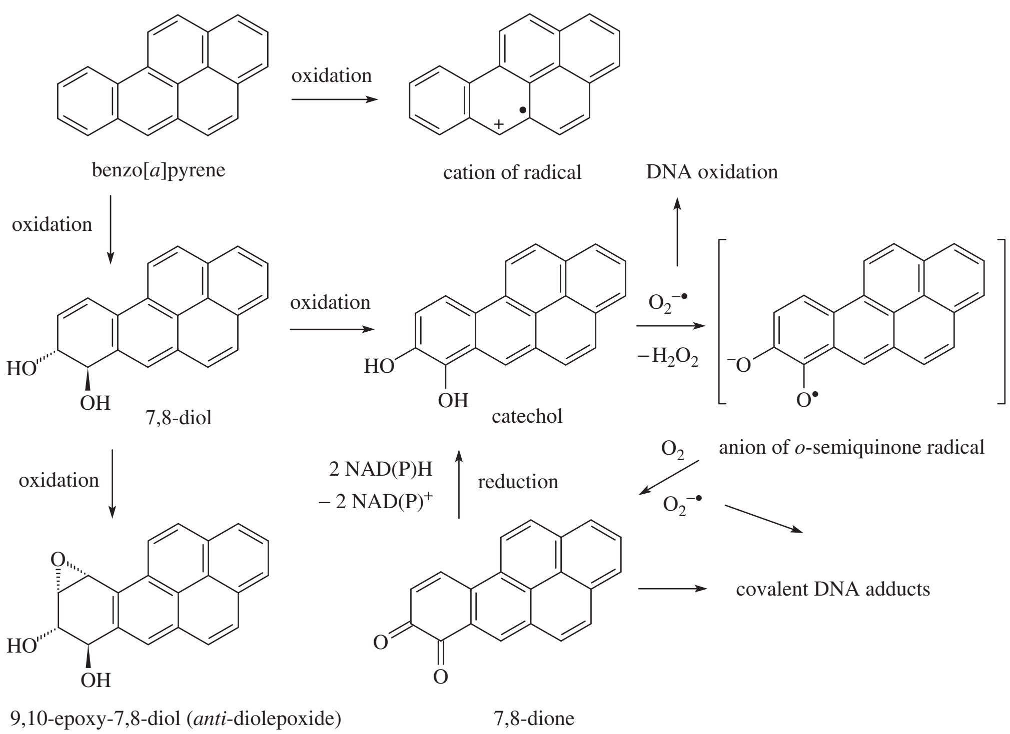


Figure 12.25 Metabolism of benzo[*a*]pyrene.

even greater carcinogenic effects. These effects are related to the metabolic transformation of PAHs to reactive metabolites, such as benzo[*a*]pyrene-7,8-diol and benzo[*a*]pyrene-7,8-dione, which can yield covalent compounds, for example with DNA (Figure 12.25). Pyrene is analogously transformed into 1-hydroxypyrene, which is the main biotransformation product. Oxidative DNA damage may similarly occur by reaction with superoxide radicals.

The metabolism of nitro derivatives of PAHs is different from metabolism of the parent PAHs. Orally administrated compounds are mainly reduced to amino derivatives by intestinal microflora. Their elimination from the body occurs after oxidation to hydroxy derivatives and acetylation of amino group in the liver. Hydroxynitro derivatives of PAHs are the major products that are resorbed unchanged from the gastrointestinal tract. Estimation of risks from dietary intake of nitro-PAH derivatives has not been worked out in detail, with respect to the limited data on their occurrence in foods. Generally, these direct carcinogens require metabolic activation. Particularly intense mutagenic effects have been demonstrated in nitro derivatives of pyrene.

12.2.5.5 Mitigation

Possibilities to limit the occurrence of exogenous PAHs are mainly connected with minimisation of anthropogenic and some non-anthropogenic sources and prevention of their dissemination into the food chain. Limitation of the formation of endogenous PAHs can often be achieved by modifying the process conditions or choosing less risky cooking processes, as discussed in the earlier section on the occurrence of PAHs in food and on the impact of technological and culinary processes on their formation.

12.2.6 Monocyclic aromatic hydrocarbons

Foods contain a variety of hydrocarbons, which arise in natural processes in the plant or animal organisms. The main hazardous substances of particular importance are, however, polycyclic aromatic hydrocarbons (PAHs). Some monocyclic aromatic hydrocarbons (MAHs) are also undesirable contaminants, particularly benzene, toluene, ethylbenzene and xylenes. Compared with PAHs, monocyclic aromatic hydrocarbons are less hydrophobic (K_{OW} values are lower) and therefore somewhat more soluble in water.

The main sources of contamination of the food chain with monocyclic aromatic hydrocarbons are oil spills, but also include a variety of coatings and plastics. Monocyclic aromatic hydrocarbons may also arise as products of the combustion of various materials. Food contamination occurs primarily by absorption from the air and water, and in animals from the surrounding environment. Another type of food contamination is migration from packaging materials. In recent years, much attention has been paid to contamination of yoghurt and biscuits by styrene (vinylbenzene) released from the polystyrene used to manufacture various packagings.

Examples of findings of monocyclic aromatic hydrocarbons in certain foods from the UK market (Table 12.17) illustrate the inputs of these substances from the external environment, because the food surface layers were generally more contaminated than inner layers. In addition to foods, the monocyclic aromatic hydrocarbons

Table 12.17 Concentrations of monocyclic aromatic hydrocarbons in selected foods.

		Content (μg/kg)		
Foods	**Sampling site**	**Benzene**	**Toluene**	**Other hydrocarbons**
Butter	Surface	<10–28	<10–275	<10–204
	Inner part	<10–15	<10–189	0–27
Cheeses	Surface	<10–18	<10–109	<10–158
	Inner part	<10–13	<10–55	<10–16
Pork lard	Surface	<10–12	10–216	<10–270
	Inner part	<10–14	<10–118	<10–18
Margarines	Surface	<10–21	12–977	<10–119
	Inner part	0–16	<10–274	0–13
Bacon	Surface	0–34	<10–180	0–44
	Inner part	<10–11	<10–10	0<10
Sausages	Surface	0–34	<10–122	<10–19
	Inner part	<10	<10–56	<10–20

content is monitored in drinking water. From a toxicological point of view, findings of benzene or styrene are particularly serious because of their carcinogenic potential.

Some MAHs may also occur in foods as technological contaminants. The study carried out in 2006–2007 in the United States, Canada and Great Britain reported on the findings of benzene in some soft drinks. The benzene concentrations in some samples exceeded 10 μg/kg (the highest amount was 90 mg/kg), which caught the attention of food safety experts. In a follow-up study conducted in the Czech Republic, 30% of samples of soft drinks exceeded the maximum limit for drinking water (1 μg/l). Benzene concentrations in these samples ranged from 1.1 to 3.2 μg/kg. In packed fermented vegetables (green olives, cucumbers and caper berries) stored under different conditions (glass or plastic containers, ambient or refrigerated storage), benzene levels higher than 10 μg/l were found in cucumbers and caper berries containing benzoic and ascorbic acids, but only when packed in plastic pouches and after prolonged storage at room temperature when ascorbic acid was partially or totally degraded during storage.

The formation of MAHs in soft drinks and some foods can be explained by decarboxylation of aromatic acids, whether natural or used as additives. The second case corresponds to the formation of benzene in soft drinks and fermented vegetables preserved with benzoic acid, which in the presence of ascorbic acid can yield benzene by decarboxylation. The reaction is catalysed by traces of transition metals. Traces of benzene found in cranberry and mango products that were not preserved with benzoic acid were due to a higher content of naturally present benzoic acid.

In comparison with other possible sources of exposure (emissions from motor vehicles, evaporation losses during handling, storage and distribution of gasoline) the benzene intake via soft drinks is negligible. Nevertheless, in order to reduce benzene intake by the body, soft drink recipes should be formulated to avoid benzene formation, which means that the simultaneous presence of benzoic acid (or its salts) and ascorbic acid should be avoided or limited.

Decarboxylation of cinnamic acid analogously yields styrene. Cinnamic acid is present as a minor constituent in cinnamon, where it arises by autoxidation of cinnamaldehyde, especially at higher temperatures. The concentration of styrene in cinnamon may even reach 40 mg/kg.

12.2.7 Nitroso compounds

12.2.7.1 Structure and nomenclature

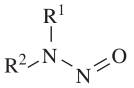
Since 1956, when the carcinogenicity of *N*-nitrosamines was proved, this group of contaminants has been given special attention. Nitroso compounds are formed from various organic compounds by the action of nitrosation reagents. The nitrosation agents may enter into food:

- during technological processing as food additives (such as nitrites or nitrates)
- as contaminants (e.g. nitrates from fertilisers)
- during drying of food by direct heating using combustion gases, which contain nitrogen oxides.

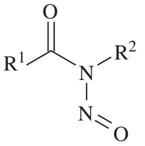
Nitroso compounds in foods may also occur as exogenous contaminants migrating from different sources, such as certain types of elastomers or latex toys, teats and soothers. Another source of nitrosamines is tobacco smoke.

All nitroso compounds contain a nitroso group (N=O) in the molecule. The most common nitroso compounds are *N*-nitroso compounds, which include *N*-nitrosamines (**12-74**) derived from secondary amines and *N*-nitrosamides (**12-75**) derived from

N-substituted amides of carboxylic acids. O-, S- and C-Nitroso compounds are also known.



12-74, N-nitrosamines



12-75, N-nitrosamides

12.2.7.2 Occurrence, main sources and dietary intake

N-Nitrosamines have been found in a number of foods. The greatest attention was paid to their occurrence in smoked meats, cheeses (especially in smoked chesses), skimmed milk, fish, beer and spirits, especially in whisky. The content of volatile and non-volatile N-nitrosamines in selected foods is given in Table 12.18 and Table 12.19, respectively.

The possibility of the formation of nitrosamines and their concentrations in foods depend on many factors, such as the presence and quantity of amines and their precursors, type and quantity of nitrosation agents, pH, temperature, reaction time, composition of food (such as fat content), method of heat treatment, presence of substances that catalyse nitrosation reaction (e.g. rhodanides and to a lesser extent chlorides), and the presence of inhibitors (e.g. ascorbic acid, its esters and salts, sulfur dioxide, phenolic antioxidants, such as gallates and melanoidins).

Table 12.18 Content of volatile nitrosamines in selected foods.

Food	Nitrosamine[a]	Content (μg/kg)	Food	Nitrosamine[a]	Content (μg/kg)
Pickled meat	NDMA, NDEA, NPYR, NPIP	traces-55	Non-fat milk powder	NDMA	0.1-3.7
Fried bacon	NPYR, NDMA,NPIP	traces-200	Fermented vegetable	NDMA, NPYR	traces-5
Microwaved bacon	NDMA, NPYR	traces-1.2	Tea	NDMA	traces-1.2
Fish	NDMA	traces-10	Alcoholic beverages	NDMA	traces-4.9
Cheeses	NDMA	traces-15	Beer	NDMA	traces-68

[a]NDMA = N-nitrosodimethylamine, NDEA = N-nitrosodiethylamine, NPIP = N-nitrosopiperidine, NPYR = N-nitrosopyrrolidine.

Table 12.19 Content of non-volatile nitrosamines[a] in selected foods.

	Content (μg/kg)				
Potravina	NPRO	NHPRO	NHMTCA	NTCA	NMTCA
Cured meat					
Cooked ham	0-40	0-100	-	-	-
Beef	70-100	240-250	130-255	328-570	0-28
Raw bacon	-	0-30	0-40	0-30	-
Fried bacon	0-20	0-80	0-100	0-50	-
Cured and smoked meat					
Mutton	230-360	350-560	160-320	960-1070	-
Sausages	0-70	-	110-410	180-210	
Raw bacon	0-20	0-60	0-1300	0-501	0-26
Fried bacon	0-40	0-90	0-2100	0-550	-
Ham	-	-	196-495	219-490	0-21
Cheeses	-	-	1062-1328	5-24	-
Duck	-	-	409-462	829-1240	35-97
Pheasant	-	-	24-75	901-1005	76-98

[a]NPRO = N-nitrosoproline, NHPRO = N-nitroso-4-hydroxyproline, NHMTCA = N-nitroso-2-(hydroxymethyl)-4-thiazolidine carboxylic acid, NTCA = N-nitroso-4-thiazolidine carboxylic acid, NMTCA = N-nitroso-2-methyl-4-thiazolidine carboxylic acid.

12.2.7.3 Formation

12.2.7.3.1 N-Nitrosamines

Nitrosamines are formed by nitrosation of secondary amines and as volatile intermediates arise by nitrosation of primary and tertiary amines and quaternary ammonium salts in acidic media. Numerous precursors of nitrosamines, such as amino compounds containing a secondary amino group, are natural components of food (amines, amino acids, amino sugars, some vitamins, lipids, aromatic substances and other compounds). For example, the amino acids proline, histidine, tryptophan and sarcosine contain a secondary amino group in the molecule. Other secondary amines are formed from amino acids by decarboxylation catalysed by enzymes, in other enzymatic reactions and by thermal decomposition at temperature about 180 °C and higher. For example, histamine, tryptamine, putrescine and agmatine arise by decarboxylation of the parent amino acids, spermidine and spermine are produced from putrescine and tryptamine yields the hormone serotonine (see Figure 10.18). Various products of the Maillard reaction also include secondary amines, such as carbinolamines, glycosylamines, aminodeoxysugars (Amadori compounds), heterocyclic compounds such as pyrroles, imidazoles, oxazolines and others. Choline bound in phospholipids yields dimethylamine by degradation (see Section 3.8.3). Other secondary amines may get into food as contaminants.

Secondary amines

The general mechanism of nitrosation of secondary amines is shown in Figure 12.26. Nitrosation reagents are formed in acidic solutions from nitrous acid and nitrites, respectively, by a sequence of reactions indicated in Figure 12.27. Nitrites are present in foods as additives or contaminants. In foods and beverages obtained by fermentation, nitrites may arise by reduction of nitrates by microbial reductases. In beer, for example, wild yeasts partly assimilate nitrates to give ammonia, and the activity of nitrate reductase

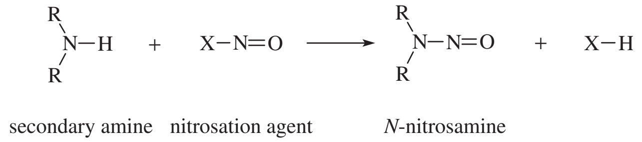


Figure 12.26 General mechanism of secondary amine nitrosation.

yields nitrites that are further reduced to ammonia by the action of ammonia nitrite reductase. Nitrates are also reduced to nitrites by some contaminating denitrifying bacteria. Nitrites are reduced to nitrous oxide (dinitrogen monoxide, N_2O) and nitric oxide (nitrogen monoxide, NO) by cytochrome cd_1-nitrite reductase, nitric oxide is reduced to nitrous oxide, from which elemental nitrogen arises by reduction, catalysed by reductases.

Effective nitrosation agents are the nitrosyl cation (NO^+), nitrogen oxides (dinitrogen trioxide, N_2O_3, and nitrogen dioxide, NO_2) or nitrosyl halogenides that are formed in the presence of hydrohalogen acids. The relative ratio or nitrosation agent depends on pH. In media with pH < 2, the predominant nitrosation agent is the nitrous acid cation ($H_2NO_2^+$) and nitrosyl cation (NO^+), respectively. At pH > 3, the main nitrosation agent is dinitrogen trioxide (N_2O_3). Both reagents are present in media with pH ranging from 2 to 5. Dinitrogen trioxide (N_2O_3) readily decomposes to dinitrogen monoxide (N_2O) and nitrogen monoxide (NO) and its dimer (N_2O_2), respectively. For example, at 25 °C and under atmospheric pressure, solutions contain equal amounts of both oxides and about 10% undissociated dinitrogen trioxide (N_2O_3). Nitrogen monoxide (NO) is also produced, together with nitrates, by disproportionation of nitrous acid:

$$3HNO_2 \rightarrow 2NO + H^+ + NO_3^- + H_2O$$

Dinitrogen monoxide (N_2O), formed by disproportionation of dinitrogen trioxide (N_2O_3), is easily oxidised by oxygen to nitrogen

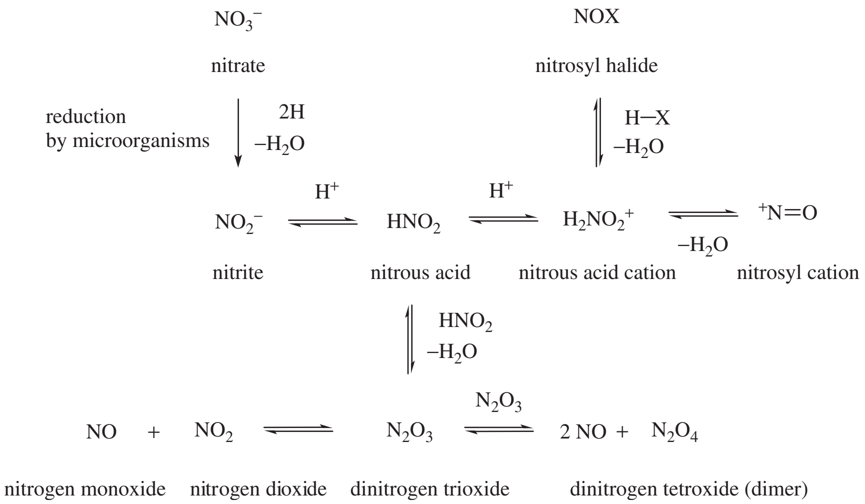


Figure 12.27 Formation of nitrosation agents in acidic media.

catalysis

$$X{-}N{=}O \quad + \quad Y^- \longrightarrow \quad Y{-}N{=}O \quad + \quad X^-$$

inhibition

$$X{-}N{=}O \quad + \quad Z \longrightarrow \text{unreactive products}$$

$$(N_2O_3 \;+\; H_2A \longrightarrow 2\,NO \;+\; H_2O \;+\; A)$$

Figure 12.28 Mechanism of catalysis and inhibition of nitrosation reaction. Y^- = catalyst (such as iodide), Z = reducing agent (H_2A = ascorbic acid, A = dehydroascorbic acid).

dioxide (NO_2):

$$2NO + O_2 \rightarrow 2NO_2$$

Nitrosation reactions may be catalysed by and also inhibited in the presence of other substances (Figure 12.28, Y–N=O is a stronger nitrosation agent than X–N=O). Catalytic effects are exhibited, for example, by thiocyanate (SCN^-) and halide (iodides, bromides and chlorides) anions. The inhibitory effects have been reported for some inorganic and organic compounds that preferentially react with nitrosation agents and reduce them. In this way ascorbic acid inhibits nitrosamine formation (at concentrations of 500–1000 mg/kg) and tocopherols (at concentrations of 100–500 mg/kg). These vitamins, if used in combination, show a higher inhibitory effect than when applied individually. Inhibitory effects are also exhibited by other substances, such as sulfur dioxide, cysteine and glutathione.

For practical reasons, *N*-nitrosamines are divided into:

- volatile nitrosamines
- non-volatile nitrosamines.

Volatile nitrosamines are a group of relatively non-polar low molecular weight compounds, while non-volatile nitrosamines are polar compounds. The most common and also the most toxic volatile nitrosamine is *N*-nitrosodimethylamine, which is produced from dimethylamine, and via dimethylamine, from other amino compounds, such as sarcosine, creatine, trimethylamine and choline (Figure 12.29). In malt, the main precursors of dimethylamine are alkaloids hordenine and gramine, occurring, for example, in germinating barley. The concentration of dimethylnitrosamine in malt and beer depends on the conditions during barley germination and malt storage. Concentrations found in malt and beers in the past have been drastically reduced by introducing indirect heating during malt kilning.

Precursors of non-volatile, but also of certain volatile nitrosamines, are often amino acids and amino acid derived amines. It is estimated that *N*-nitrosoamino acids constitute about 1% of the total *N*-nitroso-compounds present in foodstuffs. For example, *N*-nitrososarcosine arises from sarcosine and also from creatine (Figure 12.29), proline yields *N*-nitrosoproline, and its decarboxylation gives *N*-nitrosopyrrolidine. *N*-Nitrosoproline also arises from ornithine. Similarly, *N*-nitrosopyrrolidine is produced from putrescine, spermidine and also from pyrrolidine, which is the product of proline decarboxylation (Figure 12.30). By the same sequence of reactions, *N*-nitroso-4-hydroxyproline arises from 4-hydroxyproline. Lysine and biogenic amine cadaverine give rise

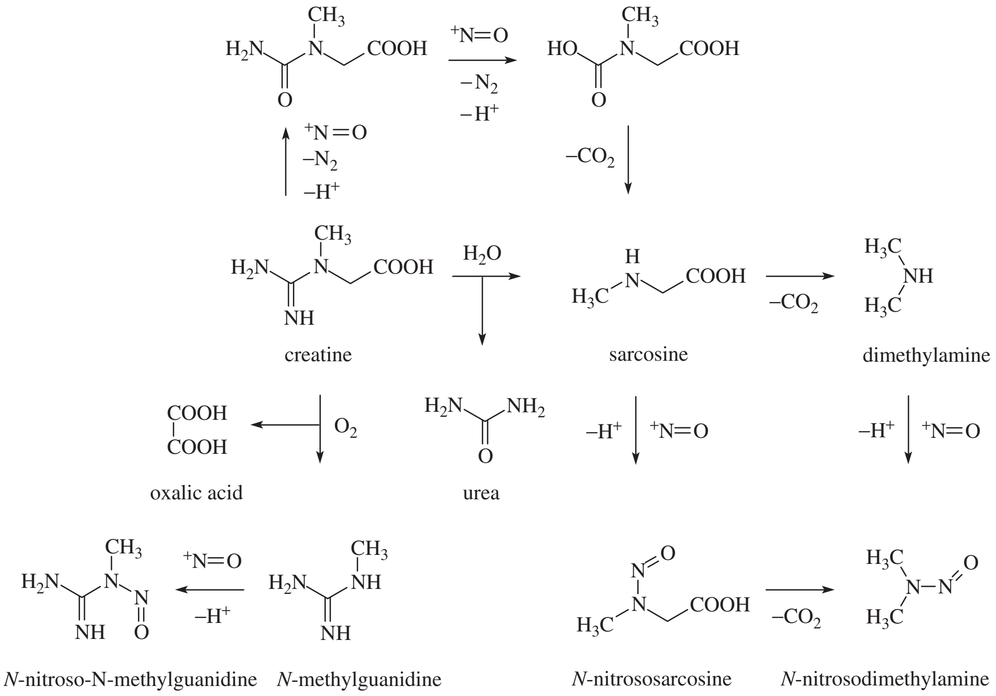


Figure 12.29 Formation of *N*-dimethylnitrosamine and other *N*-nitroso compounds from creatine and sarcosine.

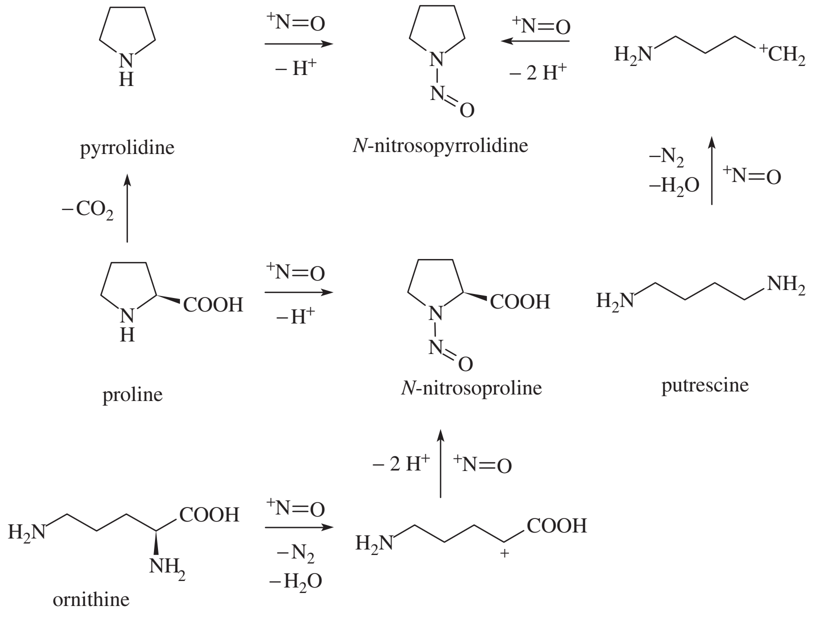
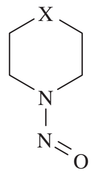


Figure 12.30 Formation of *N*-nitrosoproline and *N*-nitrosopyrrolidine.

to *N*-nitrosopiperidine. *N*-Nitrosomorpholine (**12-76**) is formed from ethanolamine, which is a component of some phospholipids. Under certain conditions, nitrosation of diethanolamine, which is used in the manufacture of personal care products, yields *N*-nitrosodiethanolamine.



12-76, *N*-nitrosopiperidine, X = CH_2
N-nitrosomorpholine, X = O

Precursors of some non-volatile nitrosamines are formed in the Maillard reaction. For example, 4-thiazolidine carboxylic acid arises from cysteine and formaldehyde (Figure 12.31), which is a component of combustion gases and liquid smoke preparations, and is also produced by the Strecker degradation of glycine and by retroaldolisation of sugars (see Section 4.7.1.2.3). Nitrosation of 4-thiazolidine carboxylic acid then yields *N*-nitroso-4-thiazolidine carboxylic acid. 4-Thiazolidine carboxylic acids substituted at C-2 arise in reactions of cysteine, with higher aldehydes resulting from the Strecker degradation of certain amino acids or in degradation of sugars. Reaction with acetaldehyde, for example, yields 2-methyl-4-thiazolidine carboxylic acid and 2-hydroxymethyl-4-thiazolidine carboxylic acid is produced in a reaction with glycolaldehyde.

Non-volatile nitrosamines are likewise produced by nitrosation of *N*-alkylsubstituted guanidines and *N*-alkylsubstituted ureas. *N*-Methylguanidine, for example, occurs in fresh meat at concentrations of up to 10 mg/kg and also arises together with *N*-nitrosoguanidine by decomposition of creatine in the presence

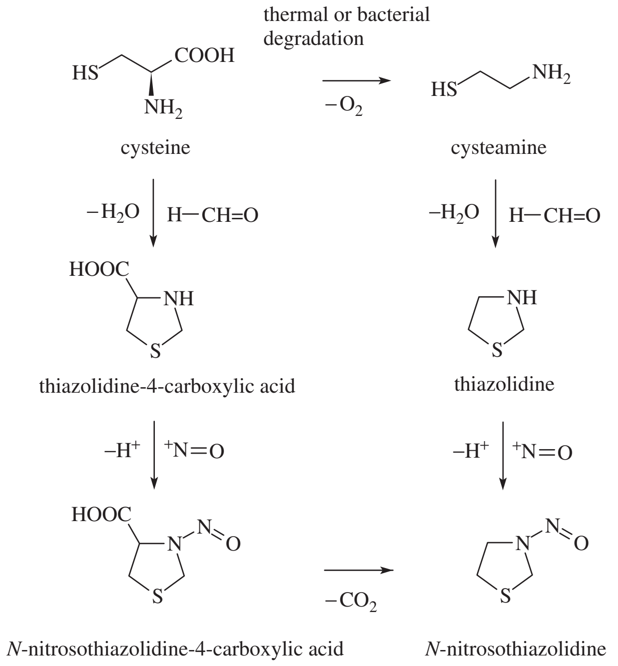


Figure 12.31 Formation of nitrosamines from cysteine.

of nitrites (Figure 12.29). Another example of *N*-substituted nitrosoguanidines is *N*-nitrosoagmatine (**12-77**) formed by nitrosation of agmatine, which is a product of arginine decarboxylation. In the meat of certain shellfish, agmatine occurs in concentrations of up to 200 mg/kg. Methyl-, propyl- and but-3-en-1-ylureas and the corresponding *N*-nitroso-*N*-alk(en)ylureas were found, for example, in fish and shellfish and ham. The key *N*-methylurea precursor is creatinine, which is present in fairly high

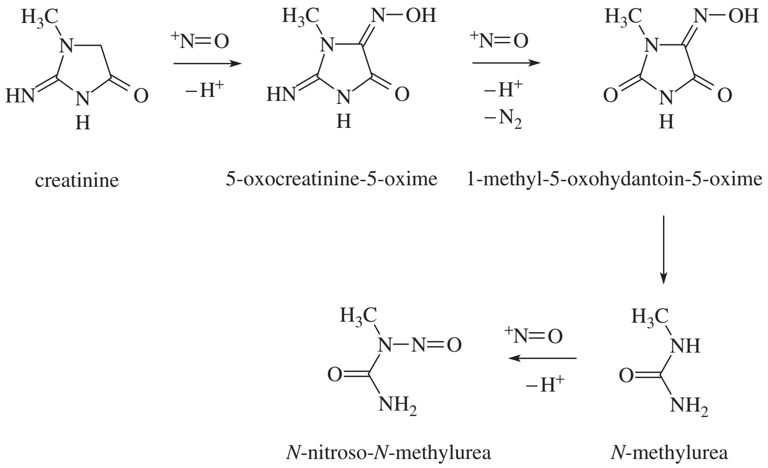
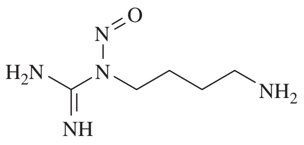


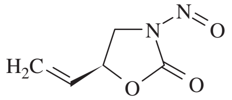
Figure 12.32 Nitrosation of creatinine.

concentrations in meat, fish and seafoods and can be nitrosated to form traces of *N*-nitroso-*N*-methylurea (Figure 12.32) via 1-methyl-5-oxohydantoin-5-oxime. The optimum pH for *N*-nitroso-*N*-methylurea formation from creatinine ranges between pH 1 and pH 3. The step from 1-methyl-5-oxohydantoin-5-oxime to *N*-nitroso-*N*-methylurea is, however, not clear; 1-methyl-5-oxohydantoin-5-oxime might be nitrosated directly to *N*-nitroso-*N*-methylurea or indirectly via the intermediate formation of methylurea. *N*-Nitroso-*N*-methylurea is a potent carcinogen that has been shown to induce cancer of various organs, mainly of the forestomach, brain and the nervous system, in a wide variety of animal species. At gastric pH, small amounts of *N*-nitroso-*N*-methylurea (0–2.6 μg/kg) were formed from various cured meats and this amount increased with additional nitrite up to 17.6 μg/kg of meat. At gastric pH, *N*-nitroso-*N*-methylurea can also be produced in other foods, such as fish sauce, herring, shrimp, sardines, oysters, mussels and pickled vegetables.

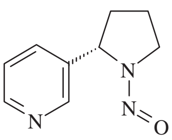


12-77, *N*-nitrosoagmatine

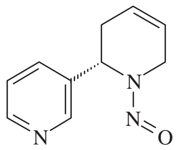
Some nitrosamines are formed only in certain commodities. For example, goitrin present in cruciferous vegetables and rapeseeds results from the glucosinolate progoitrin by the action of myrosinase, and its nitrosation yields *N*-nitroso-5-vinyloxazolidin-2-one (**12-78**). During the burning of tobacco there similarly arise, in addition to common volatile nitrosamines (*N*-nitrosodimethylamine and its higher homologues), non-volatile nitrosamines, such as *N*-nitrosodiethanolamine and nitrosamines derived from nicotine and some other tobacco alkaloids. Examples of these nitrosamines are *N*-nitrosonornicotine (**12-79**), *N*-nitrosoanatabine (**12-80**), *N*-nitrosoanabasine (**12-81**) and 4-(methylnitrosamino)-1-(3-pyridyl)butan-1-one (**12-82**).



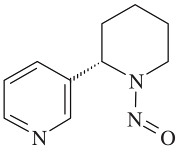
12-78, *N*-nitroso-5-vinyloxazolidin-2-one



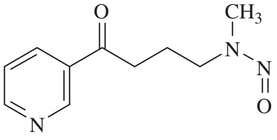
12-79, *N*-nitrosonornicotine



12-80, *N*-nitrosoanatabine



12-81, *N*-nitrosoanabasine



12-82, 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)butan-1-one

Primary amines

Nitrous acid reacts with primary aliphatic amines in the presence of mineral acids with the formation of volatile nitrosamines, which isomerise to stable diazohydroxides. Diazohydroxides are split into nitrogen and a primary alkyl cation, which may partly isomerise to a secondary cation and both cations are eliminated, which yields the corresponding alkene. Substitution reactions then yield primary and secondary alcohols and symmetrical and unsymmetrical secondary amines. In the presence of hydrohalogen acids, both alkyl

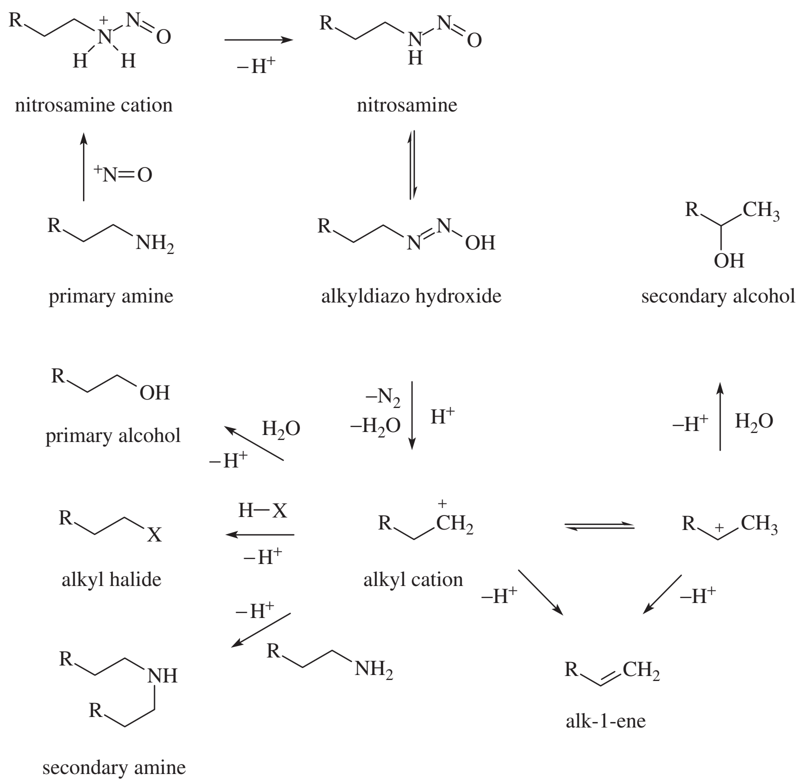


Figure 12.33 Reaction of primary amines with nitrous acid in acidic solutions.

halides are produced (Figure 12.33). Aromatic primary amines, which should however not be natural food components, yield by nitrosation diazonium salts $(Ar–N{=}N)^+ X^-$. In the absence of mineral acids, nitrous acid reacts with primary, secondary and tertiary aliphatic amines with the formation of nitrites. Secondary amines, for example, yield salts, the structure of which is $(R^1R^2NH_2)^+NO_2^-$.

Tertiary amines

Tertiary amines react with nitrous acid in the presence of mineral acids to form nitrosamine cations, which may be cleaved analogously as nitrosamines derived from primary amines to alkyl cations and the corresponding nitrosamines. Splitting of hyponitrous acid ($H_2N_2O_2$) yields the corresponding imine cation, which decomposes to an aldehyde and a secondary amine (Figure 12.34).

12.2.7.3.2 N-Nitrosamides

N-Substituted amides are produced in small amounts by heating carboxylic acids with amines. They are formed relatively easily from fatty acids or their esters (such as triacylglycerols) and amines. The reactivity of esters of fatty acids is greater than the reactivity of free fatty acids. At temperatures above 150 °C, amino acids also

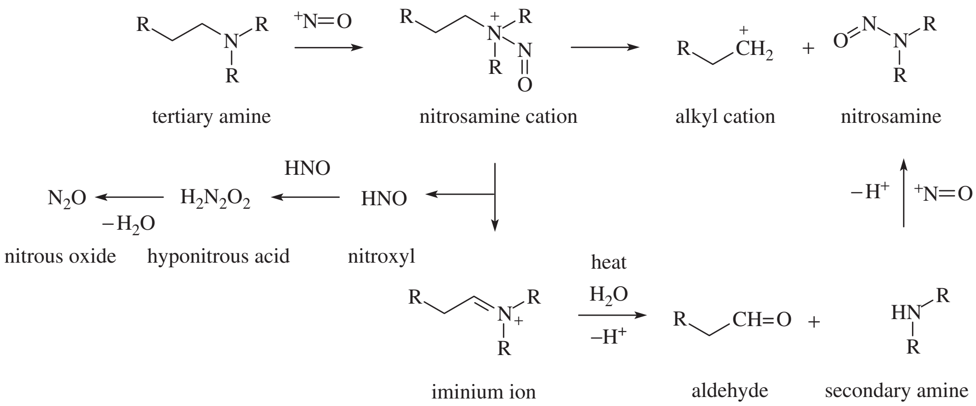


Figure 12.34 Reaction of tertiary amines with nitrous acid in acidic solutions.

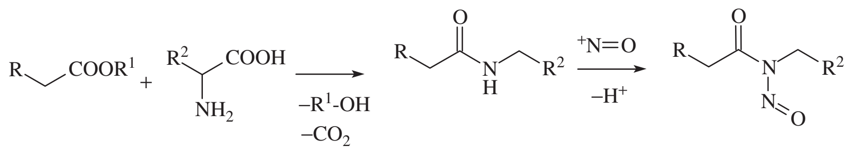
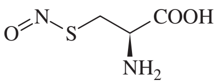


Figure 12.35 Formation of nitrosamides from amino acids and lipids.

react to form amides. Nitrosamides are formed by nitrosation of *N*-substituted amides by the same mechanisms as *N*-nitrosamines from secondary amines (Figure 12.35).

12.2.7.3.3 S-, O- and C-Nitroso compounds

In addition to *N*-nitroso compounds, *S*-nitroso compounds are also formed in foods. The reaction of nitrite with the sulfhydryl group of free cysteine or protein-bound cysteine leads to the formation of mutagenic *S*-nitrosocysteine (**12-83**), which was found in meat and meat products containing nitrites. Nitrosothiols in meat, including *S*-nitrosocysteine, consume 3–12% of added nitrite. They show antimicrobial activity, participate in the development of the characteristic aroma of smoked meat and in transnitrosation reactions. For example, they react with myoglobin and yield nitroxymyoglobin (see Section 9.2.1.5.3).



12-83, *S*-nitrosocysteine

Less information is available on the occurrence of *C*-nitroso-compounds in foods. Stable compounds are derivatives in which the nitroso groups are bound to the tertiary carbon. Such a compound is, for example, the nitrosation product 3-deoxy-D-*erythro*-hexosulose, which is one of the major degradation products of glucose and other sugars (Figure 12.36).

Compounds with a nitroso group on the primary or secondary carbon irreversibly isomerise to isonitroso compounds that are oximes of aldehydes or ketones:

$$R–CH_2–N{=}O \rightarrow R–CH{=}N–OH$$
$$R^1R^2CH–N{=}O \rightarrow R^1R^2C{=}N–OH$$

Examples of such compounds are products of nitrosation of ketones arising from autoxidation of lipids. Their oximes and oxime decomposition products are flavour-active components of smoked meat. The reaction begins at the methylene group adjacent to the carbonyl group. The elimination of a proton provides a ketone anion that reacts with a nitrosyl cation to yield a *C*-nitroso compound that isomerises to oxime (Figure 12.37).

Another example is the nitrosation of creatinine in meat, by which 5-oxocreatinine-5-oxime and 1-methyl-5-oxohydantoin-5-oxime can arise (Figure 12.32). Some phenols also react with nitrosation reagents yielding nitroso compounds and oximes. In addition to nitroso compounds and oximes, in reactions with nitrosation agents there also arise nitro, oxynitro and nitronitroso compounds, as in the case of polycyclic aromatic hydrocarbons (PAHs). The mechanisms of their formation and other aspects of their presence in food are not yet sufficiently known. An example is the nitrosation of *p*-coumaric acid, which is dependent on pH. Acidic pH results in 4-hydroxybenzaldehyde (16%), 1′,4-dihydroxybenzeneacetaldehyde oxime (59%), 4-hydroxy-1′-oxobenzeneacetaldehyde oxime (26%) and 7-hydroxy-1,2(4*H*)-benzoxazin-4-one (6%), whereas 4-(2-oxido-1,2,5-oxadiazol-3-yl)phenol was formed at acidic (6%) and neutral and alkaline pH (both 1%) (Figure 12.38).

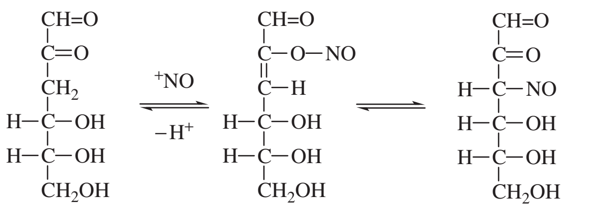


Figure 12.36 Nitrosation of 3-deoxy-D-*erythro*-hexosulose.

12.2.7.4 Health and toxicological assessment

Although food and tobacco products are important sources of external exposure to *N*-nitrosamines, exposure also occurs from nitrosamines produced internally in the digestive tract. About 5% of ingested nitrates are reduced to nitrites in saliva. These nitrites can subsequently react with secondary and tertiary amines, as well as *N*-substituted amides, carbamates and other related compounds, to form *N*-nitroso compounds within the gastrointestinal tract. This internal formation is a major source of human exposure to *N*-nitrosamines.

N-Nitrosamines exhibit mutagenic, teratogenic and mainly carcinogenic effects. Carcinogenicity has been demonstrated in various organs of a number of animals. Many nitrosamines were classified by the IARC as Groups 2A or 2B carcinogens. The synergistic effect of *N*-nitrosamines taken in with other carcinogenic compounds has also been demonstrated. EFSA are discussing the benefits of eating vegetables, especially leafy vegetables with a higher content of nitrates, and the risk of nitrosamine formation *in vivo*. In a similar context, the antimicrobial protection against bacteria *Clostridium botulinum* in certain meat products by added nitrites is being evaluated. In general, reducing the levels of nitrites to the minimum necessary amount seems to be an appropriate strategy for limiting the exposure of consumers to nitrosamines. In terms of chemical safety, it is also necessary to monitor and possibly eliminate the

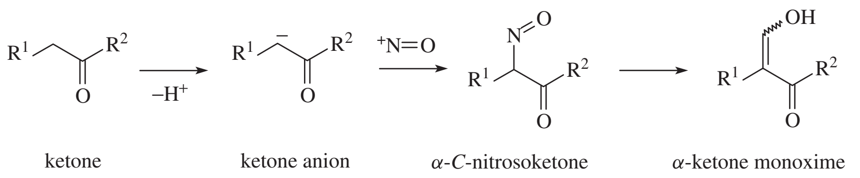


Figure 12.37 Formation of oximes from ketones.

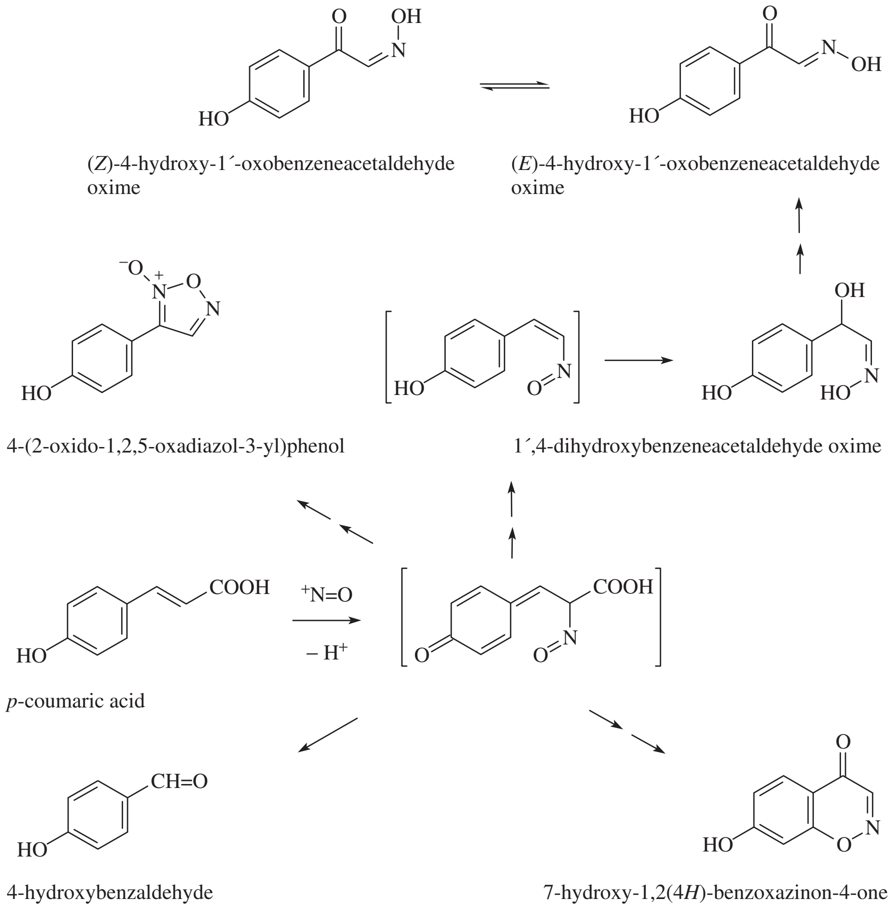


Figure 12.38 Nitrosation of *p*-coumaric acid.

migration of nitrosamines from food packaging and other contact materials.

At present, maximum levels of nitrosamines in foods are set only for beer in some EU member states, which stipulate that beer may contain up to 0.5 μg/l of *N*-nitrosodimethylamine and the sum of nitrosamines including *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodibutylamine, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine and *N*-nitrosomorpholine must not exceed 0.0015 mg/l. Action levels for *N*-nitrosodimethylamine in barley malt and malt beverages range from 5 to 10 μg/l. In other foods, nitrosamines have not been limited since 2004. Within the EU, legislation also exists for infants' teats and soothers (Commission Directive 93/11/EEC), which limits total *N*-nitrosamines to 10 μg/kg and nitrosatable materials to 200 μg/kg. Annexes II and III of the European Cosmetic Directive 76/768/EEC specify that nitrosamine contamination or *N*-nitrosodiethanolamine contamination must not be present in health and beauty care products or cosmetics in amounts exceeding 10 μg/kg.

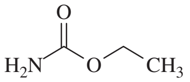
12.2.7.5 Mitigation

The formation of nitroso compounds in foods can be successfully controlled by reducing the concentration of added nitrite, drying foods by indirect heating, using substances that inhibit nitrosation (such as ascorbic acid and tocopherols) and by suitable changes to the technological processes (lowering the temperature during processing). For example, the technological modification of drying malt indirectly instead of directly by combustion gases, leads to a significant reduction in the content of volatile nitrosamines in beer.

12.2.8 Ethyl carbamate

12.2.8.1 Structure and nomenclature

The presence of toxic carbamic acid ethyl ester (ethyl carbamate, also called urethane, **12-84**) in fermented food products was first observed in 1976. The Canadian authorities first drew attention to this fact in 1985, when they carried out an extensive inspection of spirits imported from Europe. The ethyl carbamate level was restricted to 30 μg/kg in table wines, 100 μg/kg in fortified wines, 150 μg/kg in distilled spirits and 400 μg/kg in fruit brandies and liqueurs. These guidelines have been used as a reference in other countries, which do not have a specific legislation on this issue.



12-84, ethyl carbamate

12.2.8.2 Occurrence, main sources and dietary intake

Concentrations of ethyl carbamate in bread, yoghurt, soy sauce, beer or wine generally reach tens of μg/kg. Substantially higher amounts of ethyl carbamate can be encountered in alcoholic beverages, especially in spirits made from stone fruits, which in rare cases can contain thousands of μg/l of ethyl carbamate. The output from the latest EU study is presented in Table 12.20.

12.2.8.3 Formation

High amounts of ethyl carbamate in some beverages were first explained by the use of diethyl dicarbonate, which was permitted as a preservative at that time (see Section 11.2.1.2.6). Findings of ethyl carbamate in products that were not stabilised with diethyl dicarbonate highlighted a number of other mechanisms of its formation. Research in the follow-up period showed that the main

Table 12.20 Typical concentrations of ethyl carbamate in selected foods and alcoholic beverages.

Material	Total number of samples	Number of positive samples	Average amount (μg/kg)	Range (μg/kg)[a]
Foods and other products				
Bakery products	50	49	6	nd–20
Fermented dairy products	22	0	–	nd
Fermented olives	3	0	–	nd
Fermented sauces	44	28	3–4	nd–18
Sauerkraut	1	1	–	29
Vinegars	10	1	–	nd–33
Yeast extracts	1	1	–	41
Alcoholic beverages				
Beers	13	1		nd–1
Wines	17	11	10–11	nd–24
Rice wines (sake)	2	2	123	81–164
Liqueurs	4	2	45–47	nd–170
Gin	1	1	–	580
Whisky	210	196	41	nd–1000
Rum	11	10	325–328	nd–1020
Vodka	60	57	386–387	nd–2140
Brandy	42	19	123–129	nd–2100
Fruit brandy	328	281	663–667	nd–7920
Stone fruit brandy	3244	2912	848–851	nd–22 000
Various spirits	86	64	590	nd–600

[a] nd = not determined (under the limit of detection).

precursors of ethyl carbamate in fermented foods were cyanides, urea, citrulline and carbamoyl phosphate.

12.2.8.3.1 Formation from cyanides

In distilled spirits, especially stone fruit spirits, ethyl carbamate is formed via reaction of ethanol with cyanides, and correspondingly isocyanate ions that are byproducts from the degradation of cyanogenic glycosides. Cyanide ions released from cyanogenic glycosides, either enzymatically or thermally, are undoubtedly the most important dietary precursors of ethyl carbamate in distillates. Cyanogenic glycosides are substances present in many food products. Examples of cyanogenic glycosides (see **10-73**) are amygdalin and prunasin occurring in stone fruits (cherries, apricots and plums) and epiheterodendrin, which is found in barley. Cyanogenic glycosides also occur in sugar cane, but their structure has not yet been precisely described. Although it can be assumed that ethyl carbamate may be formed during the fermentation of stone fruits, the transition of ethyl carbamate to the distillate is not very significant due to its low volatility (relatively high boiling point of 185 °C). A substantial proportion of ethyl carbamate in distillates arises by photochemical reactions. The most efficient light is that with wavelengths of 350–425 nm. In addition to the amount of precursors present, light exposure, temperature, pH and the presence of certain other compounds are the main factors influencing the formation of ethyl carbamate in distilled spirits during storage and transport.

It is supposed that the main reaction is the oxidation of cyanides, released from cyanogenic glycosides, to cyanates that may isomerise to isocyanates:

$$2N{\equiv}C^- + O_2 \rightarrow 2\ N{\equiv}C{-}O^- \leftrightarrow 2\ O{=}C{=}N^-$$

The reaction is effectively catalysed by divalent copper ions, which are often released from the distillation apparatus. By another reaction copper cyanate is produced, and nucleophilic addition of water gives rise to copper carbamate, which provides ethyl carbamate by alcoholysis or decomposes to copper hydroxide, carbon dioxide and ammonia.

$$\begin{aligned}
2C{\equiv}N^- + 4HO^- + 4Cu^{2+} &\rightarrow 2N{\equiv}C{-}O^- + 4Cu^+ \\
&\quad + 2H_2O \\
Cu^{2+} + 2N{\equiv}C{-}O^- &\rightarrow Cu(O{=}C{=}N)_2 \\
Cu(O{=}C{=}N)_2 + 2H_2O &\rightarrow Cu[O(O{=}C)NH_2]_2 \\
Cu[O(O{=}C)NH_2]_2 + 2CH_3CH_2OH &\rightarrow 2CH_3CH_2OC({=}O)NH_2 \\
&\quad + Cu(OH)_2 \\
Cu[O(O{=}C)NH_2]_2 + 2H_2O &\rightarrow Cu(OH)_2 + 2CO_2 \\
&\quad + NH_3
\end{aligned}$$

Ethyl carbamate can also be produced in small quantities by other reactions, for example from vicinal dicarbonyl compounds, such as methylglyoxal, biacetyl and pentane-2,3-dione, which are byproducts of fermentation (see Section 8.2.2.1.3). Some dicarbonyl compounds may arise by fragmentation of sugars.

12.2.8.3.2 Formation from N-carbamoyl compounds

Ethyl carbamate precursors in wine, beer, yoghurt and some other fermentation products are mainly *N*-carbamoyl compounds, such as urea (carbamide) and citrulline, which are the result of catabolic processes in yeasts and bacteria from arginine, an amino acid abundantly represented in grapes. As a result of the cleavage of urea from arginine through the action of arginase in yeasts and bacteria, ornithine and urea are produced, and the product of catabolism of arginine (by arginine deiminase) in lactic acid bacteria is citrulline.

Ethyl carbamate in wine is formed (mostly at the end of fermentation) from urea. The intermediates of its degradation are probably cyanates and cyanic acid (HO–C≡N), also known as hydrogen cyanate, which may isomerise to isocyanic acid (H–N=C=O). Isocyanic acid can also arise by protonation of the cyanate anion and nucleophilic addition of ethanol to isocyanic acid yields ethyl carbamate. Isocyanic acid also reacts with other nucleophilic reagents, such as water (with formation of ammonia and carbon dioxide), thiols and amino groups of proteins. By catalysis with ornithinecarbamoyl transferase, citrulline is transformed into ornithine and carbamoyl phosphate, the ethanolysis of which yields ethyl carbamate (Figure 12.39).

Azodicarbonamide, used as a blowing agent in beer bottle cap liners and as a bread improver, has also been suggested as an ethyl carbamate precursor.

12.2.8.4 Health and toxicological assessment

Ethyl carbamate is characterised by a broad spectrum of biological effects. Even in the 1940s, ethyl carbamate was used as an anaesthetic substance, which was particularly suitable for children, as it acts on the central nervous system and has sedative effects similar to those of barbiturates. At the beginning of the 1940s, however, reports also appeared of ethyl carbamate's carcinogenicity. In 2007, the International Agency for research on Cancer (IARC) reassessed ethyl carbamate and up-graded its classification from Group 2B to group 2A.[3] The current tolerable daily intake (TDI) is 20 ng/kg body weight, which corresponds (in the case of a person weighing 70 kg) to an annual intake of 520 μg. This value could easily be exceeded in some population groups (consumers of higher amounts of alcohol).

At present, there is no international standard for the maximum allowable level of ethyl carbamate in foods. However, some countries, such as Canada, Korea and some member states of the EU (such as France, Germany and Czech Republic) have established maximum levels of ethyl carbamate in alcoholic beverages. In the Czech Republic, for example, the limit for the content of ethyl carbamate in fruit spirits is 0.4 mg/l) and for other spirits and wines the maximum level of ethyl carbamate is lower by one order of magnitude.

Toxicological studies have shown that nearly 90% of orally received ethyl carbamate is excreted as carbon dioxide by the action of cytochrome P450 oxidase complex. In the minor pathway, illustrated in Figure 12.40, however, ethyl *N*-hydroxycarbamate, vinyl carbamate and oxiran-2-yl carbamate arise as minor products,

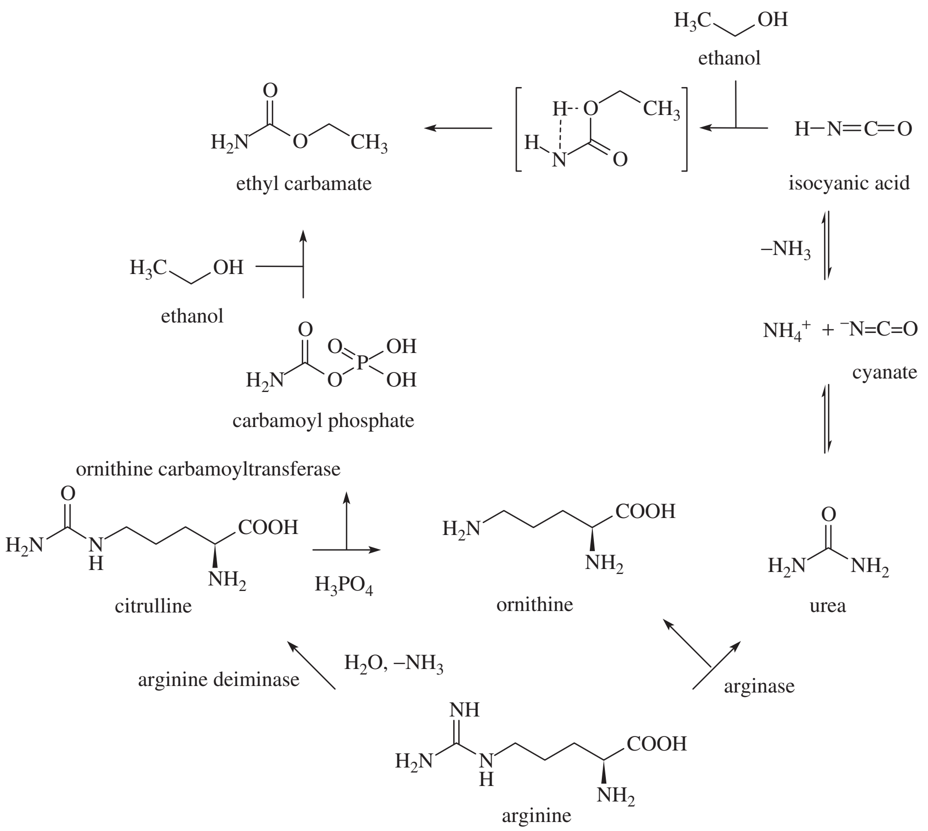


Figure 12.39 Formation of ethyl carbamate from *N*-carbamoyl compounds.

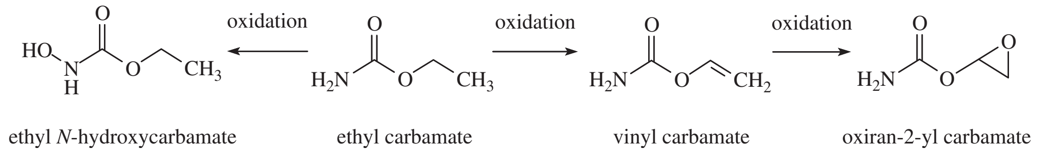


Figure 12.40 Metabolic activation of ethyl carbamate.

which are responsible for the genotoxic and carcinogenic effects of ethyl carbamate.

12.2.8.5 Mitigation

The joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated ethyl carbamate in 2005 and concluded that its intake from foods, including alcoholic beverages, would be of little concern. However, dietary exposure of ethyl carbamate from both food and alcoholic beverages was of concern, and measures to reduce its concentrations in some alcoholic beverages were recommended. Preventive measures in the production of distilled spirits (such as whisky and stone fruit spirits) are based on the reduction of the amount of cyanogenic glycosides. It is therefore recommended to use low cyanogen barley varieties, fruit which has had the stones removed, or to minimise the mechanical damage to the stones and seeds. Even for small distilleries, simple options like fruit destoning exist to minimise the ethyl carbamate content. It is also recommended that the first fraction of distillate containing greater amounts of isocyanic acid is not used. After exposure of spirits in clear glass bottles to solar radiation, maximum concentrations of urethane are reached in about 2 days, therefore measures should be taken to prevent products from light exposure, for example, by using proper containers and covering boxes. Other possibilities of reducing the levels of ethyl carbamate include special copper catalysts added to the mash or in front of the dephlegmators in conventional distillation equipment.

One possibility for the production of wines is the use of genetically modified *Saccharomyces cerevisiae* yeast with low arginase activity, which are able to metabolise urea or to use the enzyme urease that catalyses the hydrolysis of urea into carbon dioxide and ammonia. Urea cannot be used as a nitrogen source. In the case of malolactic fermentation, the use of pure *Oenococcus oeni* yeast cultures is recommended. Generally, it is recommended to keep the temperature in the range of 18–25 °C during fermentation, or to use a lower pH value, which increases during fermentation due to generated ammonia. Special care should be taken to minimise heat

exposure by maintaining the correct cold temperature, preferably at or below 20 °C, and critically not above 38 °C, along the chain from production through shipment to storage and retail.

12.3 Microbial toxins

Over the last 20 years, at least in the industrialised world, food-borne diseases caused by fungi, bacteria, parasites, viruses and prions have generated substantial media attention (such as Bovine Spongiform Encephalopathy, BSE, in cattle caused by a specific type of misfolded proteins called prions, which is a neurological disease commonly known as 'mad cow disease').

Microorganisms invading food raw materials and foods and occurring under adverse circumstances in ready-to-eat meals can cause various health problems. According to the nature of the agent and mechanism of its action, food borne diseases are divided into infections (toxo infections) and poisoning (intoxications). Studies of intestinal infectious diseases indicate that between 50 and 60% of all causative agents are unidentified. The following sections deal only with toxic metabolites of microscopic filamentous fungi known as **mycotoxins** and with bacterial toxins called **bacteriotoxins**. The inherent vegetative forms of microorganisms or their spores do not constitute a health hazard.

12.3.1 Mycotoxins

Mycotoxins are toxic secondary metabolites produced by toxinogennic fungal species (microscopic filamentous fungi, also known as moulds), multicellular, eukaryotic, heterotrophic, saprophytic or parasitic microorganisms, which, together with yeasts (estimated to be 1% of all fungal species) are a group of microscopic fungi. Microscopic fungi belong to 6000 genera with 64 000 species out of about 100 000 fungi. In medical and veterinary mycology about 150 species of microscopic pathogenic fungi are known, and in foods 114 fungal and 12 yeast species have been characterised. Of the total number of 114 species that are of importance in foods, 65 species are toxinogenic. Mycotoxins are produced by vegetative parts of fungi known as mycelia and secreted into the substrate, but can also occur in the reproductive structure – spores that contaminate the human environment. Poisoning associated with exposure to mycotoxins in food and feed is demonstrated by various symptoms, known collectively as **mycotoxicoses**. The symptoms of a mycotoxicosis depend on the type of mycotoxins, its concentration and length of exposure, as well as age, health and sex of the exposed individual. Mycotoxicoses can be categorised as acute or chronic. Acute toxicity generally has a rapid onset and an obvious toxic response. Chronic toxicity is characterised by low-dose exposure over a long time period, resulting in cancers and other generally irreversible effects. Mycotoxins, in view of their ubiquity, can occur at virtually all levels of the food chain of humans and livestock.

Of nearly 1000 known mycotoxins, more than 300 have been at least partially characterised, and approximately 20 of them can occur in food or feed in toxicologically relevant concentrations. To ensure the protection of consumer and livestock health, the maximum concentrations for these mycotoxins were set (or a determination based on available data is considered) in selected food commodities. According to FAO estimates, contamination by mycotoxins in up to 25% of food consumed can be demonstrated, and more severe levels of contamination can be found particularly in developing countries. It should be noted that the negative health effects resulting from dietary intake of mouldy food has occurred for as long as the human race has existed, especially since humans left the nomadic way of life and began to grow different crops and store surpluses. The oldest described mycotoxicosis is ergotism, induced by metabolites (alkaloids) of ascomycetes known as ergot fungi, the prominent member of which is the fungus *Claviceps purpurea* that grows on rye and related plants. Another mycotoxicosis is alimentary toxic alexia (ATA), which is induced by T-2 toxin, and related trichothecenes, produced by fungi of the genus *Fusarium* and by certain other fungi. Consumption of these mycotoxins can result in alimentary haemorrhage and vomiting, and direct contact causes dermatitis. The so-called yellow rice disease is caused by fungi of the genus *Penicillium* (e.g. *P. citreonigrum*), which produces the causative agent yellow mycotoxin citreoviridin and another mycotoxin called citrinin. The symptoms in the latter stages of this disease are characterised by hypothermia, flaccid paralysis and cardiovascular disturbances. A systematic study of mycotoxins was initiated by increased signs of dangerous mouldy food and feed in the 1960s. The major impetus for the research focused on mycotoxins was an event that took place in 1960 in Great Britain, where there was a series of mass deaths of about 120 000 turkeys and other poultry. Investigations demonstrated a correlation with the birds having been fed mouldy feed (peanut meal). The causative agents of the intoxication were identified as then-unknown fungal toxins that were called aflatoxins (from the Latin name of the fungus, *Aspergillus flavus*).

Despite intensive worldwide research on mycotoxins, many questions still remain open. Finding an effective long-term strategy to prevent or at least minimise the formation of these toxic contaminants in the process of growing and storing agricultural crops (food raw materials) is one of the key objectives of the ongoing studies. Reduction of risks to the consumer (and livestock) of dietary exposure to mycotoxins, as in the case of other pollutants, begins on the farm. Evaluation of the bioavailability and overall toxicological significance of mycotoxins and their conjugated forms can significantly contribute not only to a comprehensive assessment of health risks to humans, but also to a deeper understanding of mycotoxicoses in farm animals.

12.3.1.1 Classification

Mycotoxins are an extremely diverse group of compounds. Their relative molecular weight usually does not exceed 1000 Da. Just as is the case for other secondary metabolites, mycotoxins cannot be simply classified into groups of compounds on the basis of their chemical structure, without consideration of their occurrence, producers or the nature and intensity of the induced toxic effects. Production of mycotoxins by toxinogenic fungi is subject to a number of factors. Under certain conditions, mycotoxins may

not be produced at all, as not all strains of potentially toxic fungi are toxinogenic. A specific mycotoxin can also be produced by representatives of several genera of fungi, and two or more mycotoxins can be produced by one fungal species.

The natural criterion for classification of mycotoxins is the mechanism for the biosynthesis of their basic skeleton from primary metabolites, which are their precursors. A number of important mycotoxins are biosynthesised by the so-called polyketide pathway, specifically patulin, penicillic acid (tetraketides), ochratoxin A and citrinin (pentaketides), zearalenone (nonaketide) or sterigmatocystin and aflatoxins (decaketides). The intermediate directly involved in the biosynthesis of the polyketide is acetyl coenzyme A, which acts in the metabolism of fatty acids and other compounds. The second major pathway of biosynthesis of mycotoxins starts with mevalonic acid. From this intermediate are formed trichothecene mycotoxins containing sesquiterpenic skeletons in their molecules. For completeness, other metabolic pathways should be mentioned, which play a role in the biosynthesis of some of the less common fungal metabolites. Amino acids are biochemical precursors of cyclic polypeptides such as ergot alkaloids and others. So-called tremorgenic mycotoxins, a typical example of which is roquefortine C, are formed by the reaction of amino acids with mevalonate. Reactions similar to reactions of the Krebs cycle yield the so-called nonadrides, examples of which are rubratoxins.

The most important producers of toxicologically relevant mycotoxins are microscopic filamentous fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium*. Examples are shown in Table 12.21. Some currently monitored mycotoxins are also produced by representatives of the genera *Claviceps*, *Alternaria*, *Chaetomium* and *Sordaria*.

Table 12.21 Examples of most important mycotoxins produced by fungi of genera *Aspergillus*, *Penicillium* and *Fusarium*.

Fungus	Dominant toxic secondary metabolites
Aspergillus spp.	
A. carneus	Citrinin
A. clavanus	Patulin
A. flavus	Aflatoxins B_1 a B_2, cyclopiazonic acid
A. ochraceus	Ochratoxins, penicillic acid
A. oryzae	Cyclopiazonic acid
A. parasiticus	Aflatoxins B_1, B_2, G_1,G_2
A. tereus	Citreoviridin, citrinin, patulin
A. tamarii	Cyclopiazonic acid
A. versicolor	Sterigmatocystin, cyclopiazonic acid
Penicillium spp.	
P. aurantiogriseum	Cyclopiazonic acid, penicillic acid
P. camemberti	Cyclopiazonic acid
P. chrysogenum	Cyclopiazonic acid
P. citreonigrum	Citreoviridin
P. citrinum	Citrinin
P. commune	Cyclopiazonic acid
P. aethiopicum	Citrinin, patulin
P. griseofulvum	Cyclopiazonic acid, patulin
P. purpurescens	Ochratoxin A
P. roqueforti	Patulin
P. crateriforme	Rubratoxins
P. simplissimum	Penicillic acid
P. brasilianum	Citrinin, ochratoxin A, cyclopiazonic acid
P. crustosum	Cyclopiazonic acid, ochratoxin A
Fusarium spp.	
F. acuminatum	Diacetoxyscirpenol, monoacetoxyscirpenol, HT-2 toxin, T-2 toxin, moniliformin
F. anthopilum	Moniliformin
F. avenaceum	Moniliformin
F. chlamydosporium	Moniliformin
F. crookwellense	Deoxynivalenol, nivalenol, zearalenone
F. culmorum	Fusarin C, deoxynivalenol, nivalenol, zearalenone
F. graminearum	Deoxynivalenol, diacetoxyscirpenol, zearalenone
F. verticillioides	Fumonisins, fusarin C, moniliformin

12.3.1.2 Occurrence

Contamination of foods can occur at all stages of its production. Figure 12.41 shows the dissemination of mycotoxins in human and animal food chains and possibilities of their further fate. Contamination of food can occur especially in cases of non-compliance of requirements for safety control of fungal cultures specifically used in various food and biotechnological processes. A comprehensive list of dietary exposure sources is as follows:

- foods of plant origin in the case of preharvest primary infection and preharvest contamination of crops and products (especially cereals, oilseeds, fruits and vegetables, often dried products and nuts), contamination of final products (secondary infection);
- food of animal origin where the animals are fed contaminated feed (milk and dairy products, meat and meat products);
- food where cultural microscopic filamentous fungi (blue cheese, fermented meat products, fermented oriental soy and other plant products) are used in the manufacture;
- biotechnology products consumed as food additives, aids or supplements (microbial proteins, technical enzymes, amino acids concentrates, vitamins and other substances).

Table 12.21 (*continued*)

Fungus	Dominant toxic secondary metabolites
F. oxysporum	Moniliformin, T-2 toxin
F. poae	Fusarin C, diacetoxyscirpenol, monoacetoxyscirpenol, HT-2 toxin, T-2 toxin, zearalenone
F. sambucinum	Fusarin C, diacetoxyscirpenol, monoacetoxyscirpenol, HT-2 toxin, T-2 toxin
F. semitectum	Moniliformin, zearalenon, fusarenone-X
F. sporotrichioides	Diacetoxyscirpenol, HT-2 toxin, T-2 toxin, zearalenone
F. tricinctum	Fusarin C

Factors affecting the extent of potential (primary) mycotoxin contamination of agricultural crops under field conditions in the preharvest period are summarised as follows:

- cultivar resistance to attack by fungi;
- degree of physiological stress to which the plants are exposed (lack of minerals, small or excessive amounts of water, soil salinity, pollution, mechanical damage caused by the attack of insects or other pests);
- virulence of pathogenic fungi;
- type of mycotoxin produced (mechanism of its biosynthesis);
- phase of plant growth cycle, in which the plant was infected by fungi and the interval between the onset of a period of mycotoxin production and harvest;
- ability of plants (enzyme systems) to transform mycotoxins into non-toxic products.

An important role in terms of the intensity of mycotoxin formation is also played by climatic conditions in the preharvest period. The production of mycotoxins is promoted especially by precipitation (high relative humidity) in the later stages of crop ripening. Increased incidence of the occurrence of toxic secondary metabolites in harvested crops can be expected, especially when several factors favourable to the growth of fungi occur at the same time. In addition to fungi of genera *Fusarium* and *Claviceps* requiring relatively high humidity, cultured plants can also be infected in the period before the harvest by toxinogenic representatives of the genera *Aspergillus* and *Alternaria* and possibly likewise by fungi of genera *Chaetomium* and *Sordaria*. Even in the preharvest period, during transport, processing or storage, contamination of plant products is also possible. Producers of mycotoxin in this case can be, in particular, fungi of genera *Aspergillus* and *Penicillium*, which can grow with lower water activity in the environment. The following aspects are crucial:

- **biological properties** of the given organism, presence of competitive microflora and inoculum size;
- **chemical properties** of the substrate, its composition, presence of substances with fungicidal activity;
- **physical properties**, temperature, water activity, atmospheric composition, adequate access of light.

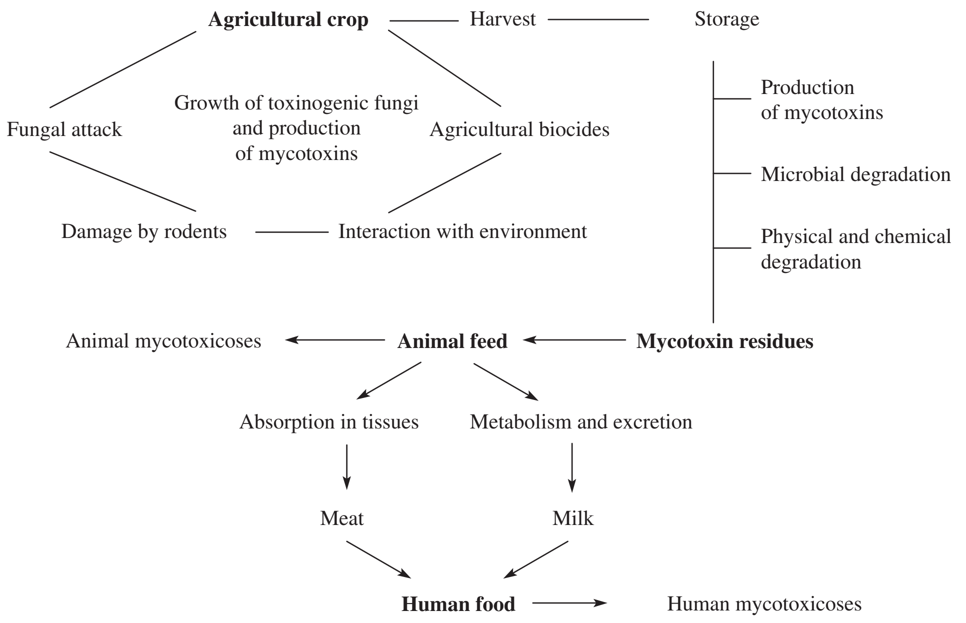
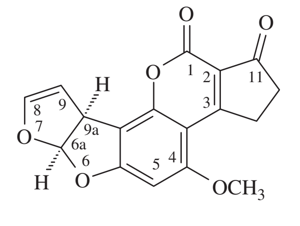
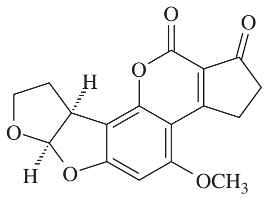


Figure 12.41 Factors influencing occurrence of mycotoxins in foods and feeds.

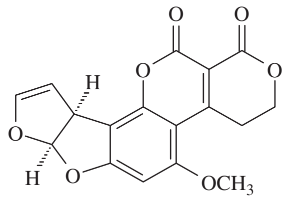
A critical factor limiting the growth of fungi during storage of agricultural crops is the content of available water. The outer layers of grains are a natural barrier to fungi. If the layers are damaged during harvest, transport or processing, mould spores can easily access the necessary nutrients for their growth, which are contained in the inner parts of the grains. Generally, smaller cereal grains (such as wheat, barley or rice) are less often attacked by fungi than larger grains, such as maize.



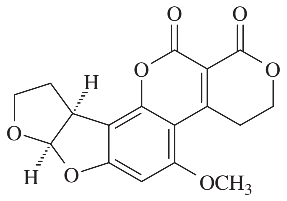
12-85, aflatoxin B_1



12-86, aflatoxin B_2



12-87, aflatoxin G_1



12-88, aflatoxin G_2

12.3.1.2.1 Major mycotoxin groups

Mycotoxins in the following sections are not sorted by their producers, but according to their structures related to toxic effects. It is obvious that some toxic secondary metabolites are produced by several types of filamentous fungi (Table 12.21).

Aflatoxins

Aflatoxins belong to the most often monitored mycotoxins, because of their high toxicity. The most important producers of aflatoxins are two closely related fungi of the genus *Aspergillus*, *A. flavus* and *A. parasiticus*. A rarer producer of aflatoxin is *A. nomius*. Their mycelia grow, under favourable conditions (temperatures between 13 and 37 °C and water activity <0.82) on virtually any substrate; however the highest amounts of aflatoxins (in some cases hundreds of thousands of mg/kg) were observed in maize, peanuts, pistachios, Brazil nuts, cottonseed and copra. Lower levels of aflatoxins are found in almonds, pecans, walnuts, raisins, figs and various spices. As evidenced by the Rapid Alert System for Food and Feed (RASFF), aflatoxins represent the contaminants most often found in the control of food imported into the EU (most often nuts and nut products). In 2007, of 754 notifications of aflatoxins in RASFF, 556 were related to nuts and nut products. In 2008 there was a further increase to 910 notifications, of which 710 were related to nuts. Also of concern is the increasing proportion of contaminated cereals, which are key items in the food basket in Europe and many other areas. This negative trend may, *inter alia*, indicate the impact of climate change (global warming) and the related development of pathogenic fungi, which require higher temperatures for their growth.

Currently 16 natural aflatoxins are known. Formulae **12-85** to **12-88** show the four major naturally occurring representatives of the B and G groups of aflatoxins, which are known as aflatoxins B_1, B_2, G_1 and G_2. The designation B (blue) or G (green) is associated with fluorescence of aflatoxins under UV light. While *Aspergillus flavus* produces mainly aflatoxins B_1 and B_2, contamination by *A. parasiticus* leads to aflatoxins G_1 and G_2 in the contaminated substrate. The basis of skeletons of all aflatoxins is coumarin condensed with bisdihydrofurofuran (dihydrobisfuran) and cyclopentanone in the first two aflatoxins, while the other two substances contain 5,6-dihydropyran-2-one instead of cyclopentanone.

Aflatoxin B_1, the chemical name for which is (6a*R*,9a*S*)-2,3,6a,9a-tetrahydro-4-methoxy-cyclopenta[*c*]furo[3′,2′:4,5]furo[2,3-*h*]-benzopyran-1,11-dione (**12-85**), is the main representative of this group of mycotoxins in contaminated food. Their total content (the sum of the four major aflatoxins) is on average higher by 24%, but the variability for different categories of foods is large (2–70%). Pistachios and Brazil nuts have a distinctly different spectrum of aflatoxins, with significantly higher average findings. Distribution of aflatoxins in contaminated commodities is very uneven, with the highest concentration often found in foci of high humidity, or in areas of mechanical damage to the plant material. Discoveries of aflatoxins have a distinct log-normal distribution, with a large number of positive findings in the area of low concentration and a small proportion of extremely contaminated samples.

Similarly to other xenobiotics, in animal organisms biotransformation of aflatoxins proceeds in three main stages (mainly in the liver): bioactivation, conjugation and deconjugation. Phase I of the biotransformation leads, apart from the formation of relatively non-toxic products, to toxic intermediates. The toxic intermediate of aflatoxin B_1 is *exo*-8,9-epoxide. Phase II represents detoxification, such as hydroxylation of aflatoxin B_1 to aflatoxins B_{2a}, M_1 and Q_1 and demethylation to aflatoxin P_1, which is accompanied by significantly reduced toxicity. These and other aflatoxins were identified as metabolites of aflatoxins of the B and G groups in the body of humans and animals and in tissue cultures. Also important is a product of oxidation called aflatoxicol. Phase III proceeds under the activity of intestinal microflora and may lead to the re-absorption of the released aflatoxin. The scope of these changes and the related toxic effects differ considerably between individual species (Figure 12.42). *Exo*-8,9-epoxide of aflatoxin B_1 reacts with DNA to form a covalent product with N^7-guanine (**12-89**) and forms an adduct with glutathione, which are excreted in the urine, and reacts with amino acids in proteins with the formation of imines and is hydrolysed by water to 8,9-dihydroxyaflatoxin, essentially 8,9-dihydroxyaflatoxin B_2, which forms adducts with proteins (as well as aflatoxicol) and is partly excreted in the urine.

When feed contaminated with aflatoxins B_1 and B_2 is given to cows, aflatoxins M_1 and M_2 resulting from hydroxylation of the parent compounds may be found in milk in roughly 12 h. The transition factor, which is the ratio between the amount of ingested precursor (aflatoxin B_1) and excreted aflatoxin M_1, is in the range of 100 : 1 to 300 : 1. The major part of this metabolite is excreted in the urine. Trace amounts of aflatoxin M_1 also appear in milk after

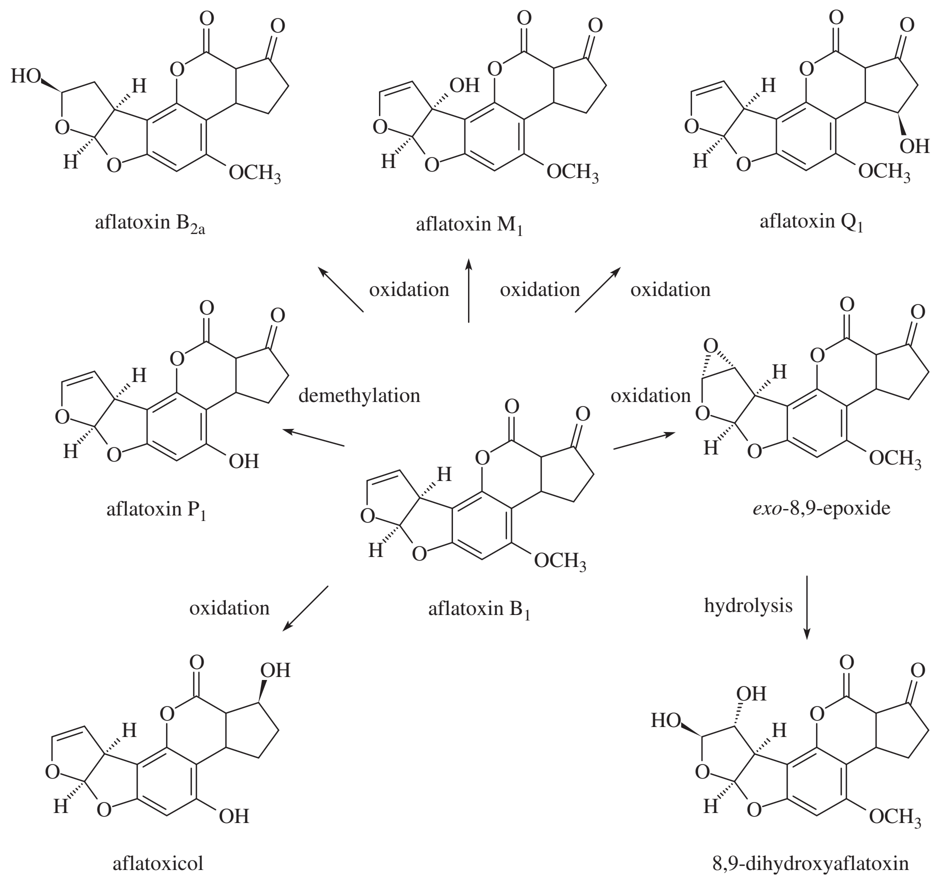
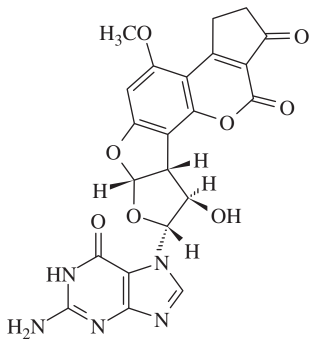


Figure 12.42 Aflatoxin B_1 metabolites.



12-89, adduct of aflatoxin B_1 with guanine

a single intake of contaminated feed, even after 2–3 days. Transient factors of aflatoxins to muscles are low in cattle and for aflatoxin B_1 are in the range of 1000–14 000. Somewhat lower values (higher risk of the occurrence of residues) were found in the livers of pigs and poultry, but foods of animal origin (except milk and milk products) do not generally represent a significant dietary source of aflatoxins.

During milling of wheat grains, aflatoxins, found mostly in the outer layer (aleurone layer) of the grain and in the germ, are redistributed among individual fractions obtained depending on degree of milling, known as the flour extraction rate. The decrease of contamination in the 85% straight run wheat flour (see Section 5.6.7.3), by about 15% of the original amount, is accompanied by higher aflatoxin concentrations in the bran. Aflatoxins are relatively hydrophilic compounds and in the production of vegetable oils they are concentrated in oilseed meals that may become a source of contamination of the food chain when used for livestock feeding. Similarly, separation of fat during milk processing, increases the content of aflatoxin M_1 in some products, such as low-fat milk, cottage cheese, whey, buttermilk and other products. Fermentation processes lead to a small decrease in the amount of aflatoxins, but, for example, on average 20–25% of aflatoxins contained in barley are transferred to beer. The highest losses occur in the final stage of the brewing process when, after boiling with hops, proteins, any remaining hops and other insoluble particles are separated by filtration. The decrease of aflatoxin concentration may be related to reactions of toxins with organic molecules, particularly with the formation of complexes with proteins, which are removed. In the production of spirits, aflatoxins from contaminated material do not pass to the distillate. Aflatoxins are relatively stable compounds therefore thermal processes do not lead to their complete elimination in materials with low moisture content. The extent of their degradation depends on the amounts of water, fat and other components. Coffee roasting at 180 °C for 10 min results in only 50% decrease of aflatoxin B_1 concentration. Other examples are shown in Table 12.22.

Table 12.22 Changes in aflatoxin B_1 and M_1 levels during processing of contaminated materials.

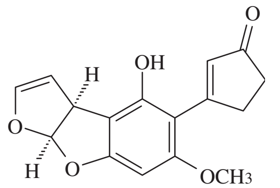
Product	Processing conditions	Losses (%)	Product	Processing conditions	Losses (%)
Peanuts	Roasting, 150 °C, 30 min	20	Rice	Steaming	0
	Dry roasting	31		Boiling under pressure, 120 °C	27
	Roasting in oil	35		Regular cooking	51
Peanut products	Roasting, 204 °C	50–60	Milk	Pasteuristion, 72 °C, 45 s	35
Peanut oil (unrefined)	Heating, 120 °C, 10 min	0		Sterilisation, 115 °C	19

Exposure of humans and animals (depending on dose) to aflatoxins may cause a variety of acute and chronic diseases. Aflatoxin B_1 is considered the most potent known natural hepatocarcinogen. One of the most tragic cases of acute human aflatoxin poisoning was reported in India in 1974, when the consumption of mouldy maize afflicted about 1000 people, about 100 of whom died. Based on the evaluation of aflatoxin concentrations and estimates of aflatoxin exposure dose, it was considered that the LD_{50} value for humans is somewhat higher than for the most sensitive animal species, such as dogs, where the LD_{50} value for aflatoxin B_1 is in the range of from 0.35 to 0.50 mg/kg body weight. Experts mention the synergies of aflatoxins and hepatitis B viruses. Numerous independent epidemiological studies have pointed to the high incidence of liver cancer in the populations in some regions of Eastern Asia and Africa, which correlates with a high dietary intake of aflatoxins. The most obvious correlation was found in areas with a frequent occurrence of chronic hepatitis B. Mutagenicity and carcinogenicity of aflatoxins generally follow the order $B_1 > G_1 > B_2 > G_2$ and are related to the interaction of reactive metabolites with DNA and inhibition of its replication and transcription, and inhibition of other processes, such as RNA and protein biosynthesis. In addition, aflatoxins induce a number of other adverse biological responses in humans, such as the so-called Reye's syndrome (a potentially fatal disease that causes detrimental effects to many organs), respiratory diseases, chronic gastritis, mental disability of children and immunosuppression. According to the IARC, aflatoxins are classified as Group 1 carcinogens to humans.[3]

In livestock, the consumption of aflatoxins in feed leads to a number of problems, which are then reflected in negative impacts on performance. Chronic exposure to aflatoxins leads to reduced growth and greater susceptibility to infections. This condition is often referred to as total failure to thrive. An extreme consequence of giving animals contaminated feed is lethal acute poisoning.

In relation to the prevention of the occurrence of aflatoxins, inhibitors of their formation are often studied. In addition to compounds used in practice for their fungicidal effects, such as preservatives, inhibitory effects of zinc or caffeine have also been demonstrated. Aflatoxin production can be stimulated by long chain fatty acids and inhibited by oleic, linoleic and lauric acids.

Contamination of plant materials with mycotoxins can become a significant economic problem, if it concerns a large volume of biomass. Because of the high carcinogenic potential of aflatoxins, special attention is paid to the possibilities of detoxification of contaminated materials, which would at least partially facilitate their use. The most common way is washing with ammonium hydroxide, often at elevated temperature and pressure. The efficiency achieved under optimal conditions can reach as high as 99%, but the principle of such treatment has not yet been satisfactorily explained. Among the degradation products of aflatoxin B_1, in model experiments, a decarboxylation product aflatoxin D_1, (3a*S*,8a*R*)-3-[3a,8a-dihydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran-5-yl]-2-cyclopenten-1-one (**12-90**) has been identified. However, decontamination may cause a number of negative changes in the composition of the treated material, which results in a decrease of its nutritional or sensory quality. The use of decontaminated products for human nutrition is prohibited. Alternatively, the contaminated feedstock may be used for the production of ethanol or other fermentation products.



12-90, aflatoxin D_1

Owing to the high toxicity of aflatoxins, maximum amounts (at a strict level for what is reasonably achievable by following good agricultural and manufacturing practices) have been set for aflatoxin B_1 and for the sum of aflatoxins B_1, B_2, G_1 and G_2 as well as for aflatoxin M_1 in milk and certain milk products. The situation is quite complicated, because the limits in Europen Commission Regulation (EC) No. 1881/2006 have been subjected to rapid change, as seen for example in Commission Regulation (EC) No. 1126/2007, since for many countries strict limits can bring about serious economic difficulties. This concerns aflatoxins and *Fusarium* mycotoxins (the problematic crop is maize). To illustrate the maximum amounts set for mycotoxins, Table 12.23 summarises typical values of different EC documents. In the regulation of mycotoxins in foods, further development can be expected based on the results of toxicological evaluation and monitoring of real levels of mycotoxins in foods, depending on the geographical origin and climatic conditions. Last but not least, influences of

Table 12.23 Examples of typical limits for mycotoxins in food.

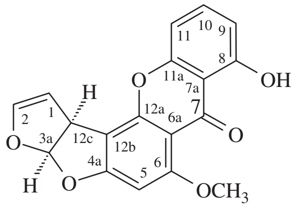
Mycotoxins	Product	Maximum levels (μg/kg)[a]
Sum of aflatoxins B_1, B_2, G_1 and G_2	Nuts, dried fruits, cereals, spices	4.0–15.0
Aflatoxin B_1	Nuts, dried fruits, cereals, spices, foods for infants and young children, dietary foods for special medical purposes	0.1–8.0
Aflatoxin M_1	Infant formulae and follow-on formulae, dietary foods for special medical purposes	0.025–0.05
Ochratoxin A	Cereals, dried vine fruit (currants, raisins and sultanas), coffee, wine, foods for infants and young children, beer, cacao, meat products, spices	0.5–10
Patulin	Apple juice and drinks from apples, cider, compotes, purees, baby food	10.0–50.0
Deoxynivalenol	Cereals, flour, pastries, biscuits, cereal snacks and breakfast cereals	200–1750
Zearalenone	Cereals, flour, pastries, biscuits, cereal snacks, breakfast cereals, baby food	20–350
Fumonisins	Maize and maize products	200–4000
T-2 toxin and HT-2 toxin	Not yet defined	–

[a]Maximum level can vary in the given range depending on the exact specification of food and depending on whether it is consumed directly or further processed. For example, the EU limit for patulin is set to 50 μg/kg in both apple juices and ciders, to 25 μg/kg in solid apple products and to 10 μg/kg in products for infants and young children.

processing technologies and transformation products of mycotoxins will be also taken into consideration, such as their conjugates with saccharides.

Sterigmatocystin

Sterigmatocystin (**12-91**) is a toxic secondary metabolite of microscopic filamentous fungi, especially fungi of the genus *Aspergillus*. One of its more prominent producers is fungus *A. versicolor*, but sterigmatocystin is also produced by fungi *A. flavus* and *A. parasiticus*. The presence of sterigmatocystin (along with aflatoxins) has



12-91, sterigmatocystin

been demonstrated in mouldy cereals, coffee beans, some animal products, such as ham, sausages and hard cheeses, where it was located mainly on the surface.

Sterigmatocystin containing the bisdihydrofurofuran skeleton is related to aflatoxins and is even regarded as their precursor. It is a xanthen-7-one derivative, with the systematic name (3a*R*,12c*S*)-8-hydroxy-6-methoxy-3a,12c-dihydro-7*H*-furo[3’,2’:4,5]furo[2,3-c]xanthen-7-one.

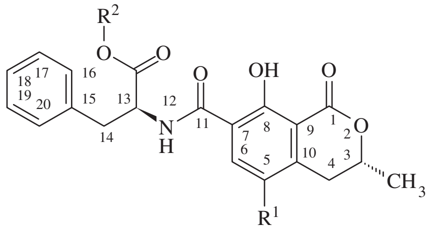
Sterigmatocystin is very acutely toxic to humans and animals and like aflatoxins acts as a hepatocarcinogen. According to the IARC, it is classified as a Group 2B carcinogen to humans.[3] As one of the priorities in the dietary risk assessment, the European Food Safety Authority (EFSA) identified the need to gather a more complete set of data on the occurrence of this mycotoxin in foods. Maximum levels in foods are not currently set due to a lack of data from which to assess the associated health risks.

Ochratoxins

Ochratoxins are a group of mycotoxins discovered in South Africa in 1965 during investigations on toxic filamentous fungi isolated from agricultural crops. The most important producers of these mycotoxins are filamentous fungi of the genus *Aspergillus*, in particular *A. ochraceus*, but some industrial strains of *A. niger* also produce these mycotoxins. In the colder climates of Europe (especially in Scandinavia), important producers of ochratoxins are fungi of the genus *Penicillium*, mainly *P. verrucosum*, *P. nordicum* and *P. carbonarius*.

From a toxicological point of view, the most important representative of this group of mycotoxins is ochratoxin A, (3*R*)-*N*-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*-2-benzopyran-7-yl)carbonyl]-L-phenylalanine (**12-92**), the molecule of which phenylalanine *N*-substituted with a derivative of (3*R*)-3,4-dihydro-3-methylisocoumarine that contains at C-5 a chlorine atom to which are attributed the toxic effects of ochratoxin A. The incorporation of the chlorine atom into the skeleton of ochratoxin A is done by the action of chloroperoxidase; the chlorine donor is inorganic chloride. Ochratoxin B (**12-92**) differs from ochratoxin A only in the absence of the chlorine atom, while ochratoxin C (**12-92**) is an ethyl ester of ochratoxin A. Ochratoxins α, β and γ resulting from from parent compounds by the loss of phenylalanine caused by peptide bond hydrolysis, are virtually non-toxic, in addition to ochratoxin B, and are not routinely monitored.

Concentrations of ochratoxin A in cereals rarely exceed 50 μg/kg, but improper storage may cause a massive increase in contamination. In addition to cereals and cereal products, ochratoxin A



12-92, ochratoxin A, $R^1 = Cl$, $R^2 = H$
ochratoxin B, $R^1 = H$, $R^2 = H$
ochratoxin C, $R^1 = Cl$, $R^2 = CH_2CH_3$

has also been identified in some legumes, coffee, cocoa, grapefruit juices, raisins, nuts, spices and wine, especially red wine, which acquires ochratoxin A from the grape skins. During coffee roasting, ochratoxin A is isomerised and degraded by up to 90% to (3*R*)-isomer and 14-decarboxyochratoxin A, respectively. The occurrence and concentration of ochratoxin A in wines is likely to increase in the southern wine regions of Europe, which is connected with the infection of grapes by toxinogenic filamentous fungi *Aspergillus carbonarius* and other species (*A. niger*), which are almost absent in the northern wine regions. Attention must also be paid to the strains of *A. niger*, which are commonly used in biotechnology for the production of a variety of hydrolytic enzymes and organic acids, particularly citric acid. Their ability to produce ochratoxin A was discovered in the 1990s. Conjugated forms were found in cell cultures of wheat and maize: methyl ester of ochratoxin A and methylester and glucoside of hydroxyochratoxin A. Ochratoxin A is a relatively stable compound (Table 12.24) and losses under normal processing conditions are not significant.

Ochratoxin A is probably the only mycotoxin whose dietary intake is mainly associated with foods of animal origin. If contaminated feed is fed to animals, ochratoxin A is correspondingly found in the bodies of farm animals; it accumulates mainly in the kidney. In pigs, concentrations of ochratoxin A in the kidney may reach hundreds of mg/kg. The transient factor is in the range of 20–60 in the kidney and between 400 and 660 in the liver. Trace concentrations of ochratoxin A were also detected in the flesh, and occasionally in cheeses with moulds on the surface and in cured salami with casings treated with edible mould cultures.

The most serious biological effects of ochratoxin A to exposed animals is nephrotoxicity, but its hepatotoxicity, genotoxicity and immunotoxicity have also been demonstrated, and its carcinogenic potential is under discussion. When contaminated feed is fed to livestock, their growth is slow and relates, *inter alia*, to lower utilisation of nutrients. In humans, exposure to ochratoxin A is associated with Balkan endemic nephropathy (BEN), also known as Danubian endemic familial nephropathy, which is a chronic kidney disease that affects the kidney and leads to fibrosis and decreased kidney function. It also is characterised by a high frequency of urothelial cancer (cancer of the urinary tract).

During research focused on ochratoxin A, the frequent occurrence of this mycotoxin was found in blood plasma of people from different parts of Europe, which emphasised the need for systematic monitoring in the human food chain (after entering the human body ochratoxin A is very persistent). The highest levels of ochratoxin A in Europe were found in the population of Denmark. Recent evaluation of dietary exposure to ochratoxin A conducted by EFSA indicates that the intake of an adult European consumer is in the range of 15–60 ng/kg body weight per week, which does not exceed the Tolerable Weekly Intake (TWI), which is, according to expert estimates, 120 ng/kg body weight. Hygienic limits for ochratoxin A residues exist in many countries, examples of which are given in Table 12.23. Maximum levels are exceeded only in exceptional cases.

Patulin

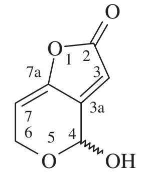
Initially patulin was evaluated as a pharmacological agent due to its antimicrobial properties. However, the gastrointestinal and dermal irritation observed in human trials prevented its use as a pharmacological agent. The most important producers of patulin are microscopic fungi of the genus *Penicillium*, especially *P. patulinum* and *P. expansum*, which are common pathogens of many fruits and vegetables. The production of this mycotoxin was also detected in some fungi of the genera *Aspergillus* and *Byssochlamys*. Within the food industry, patulin contamination is considered of greatest concern in apples and apple products, which are the main sources of human patulin intake. Nevertheless, this mycotoxin has also been found at significant concentrations in other fruits, such as pears, peaches, strawberries, blueberries, cherries, apricots, grapes and in cheeses and animal feed, especially in silages. Patulin is a relatively common contaminant of fruit concentrates and juices, especially when overripe or damaged fruit has been used. Its concentrations do not normally exceed 0.1 mg/kg, but can reach about 2500 μg/kg if the juice is obtained from rotten apples, which may contain up to about 45 000 μg/kg of patulin.

Patulin, 4-hydroxy-4*H*-furo(3,2-*c*)pyran-2(6*H*)-one (**12-93**) is a furopyrone mycotoxin, which occurs in nature as a racemate.

Patulin is highly soluble in water and is relatively stable in acidic media (pH range from 3.0 to 6.5); therefore special attention is devoted to reduction of its content in foods. The assimilated

Table 12.24 Changes in the content of ochratoxin A during processing of contaminated crops.

Products	Processing conditions	Losses (%)	Products	Processing conditions	Losses (%)
Coffee beans	Roasting, unspecified	10–20	Brewing mash	Cooking	27–28
Coffee beans	Roasting, 200 °C, 5 min	0	Cereal products	Autoclaving, 120 °C, 3 h	30



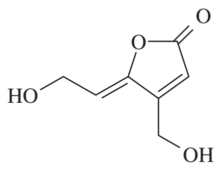
12-93, patulin

information and recommendations can be summarised in the following points:

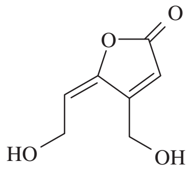
- removing mycelium from the product, even with the adjacent biomass, is not sufficient for decontamination, because patulin easily diffuses into the whole material;
- cold juice pressing does not have a significant impact on the content of patulin in the final product;
- patulin present in apple pomace, a byproduct of juice production, may pose a risk to livestock if used as feed;
- storage of products containing patulin (especially at higher temperatures) leads to a gradual decrease of its content;
- thickening of juices by vacuum distillation reduces patulin content by an average of 25%;
- pasteurisation at 80 °C leads to a negligible reduction in the concentration of patulin, but sterilisation may reduce levels of patulin in apple juice by about 20% or more;
- decrease of patulin content in foods (to about 40–95% of the original concentration) probably occurs during microwave heating.

Patulin is virtually absent from wines, because fermentation of contaminated juices by commercial yeast *Saccharomyces cerevisiae* (also by other microorganisms, such as *Gluconobacter oxydans*) leads to relatively rapid transformation of patulin into ascladiols, 5-(2-hydroxyethylidene)-4-hydroxymethyl-5*H*-furan-2-ones (**12-94** and **12-95**), but practically no information exists about the toxicity of these diols. Sulfites and compounds containing sulfhydryl functional groups (cysteine and glutathione) can contribute to a significant acceleration of patulin degradation. Owing to the electrofilicity of patulin, reactions with other nucleophilic substances such as amino acids (especially lysine and histidine) or proteins can be expected. Increased patulin degradation induced by hydroxyl and other free radicals was observed in fruit juices fortified with ascorbic acid to the level of 500 mg/l.

In addition to the antibiotic effects of patulin against Gram-negative and Gram-positive bacteria, its antifungal and antiviral properties (inhibition of replication of mycoviruses) have also been reported. Its carcinogenic and mutagenic effects have subsequently been proven, associated with inhibition of RNA transcription and selective DNA damage. Also demonstrated were negative effects on the gastrointestinal tract and neurotoxic and immunotoxic effects,



12-94, (*Z*)-ascladiol

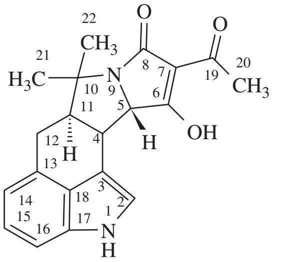


12-95, (*E*)-ascladiol

therefore the content of patulin, a Group 3 carcinogen (a compound for which there are not enough data to allow its classification)[3] in food should be reduced to a minimum technically achievable level. The limits set in the EU for apples and apple products are summarised in Table 12.23.

Cyclopiazonic acid

Cyclopiazonic acid or α-cyclopiazonic acid, by its chemical name (6a*R*,11a*S*,11b*R*)-*rel*-10-acetyl-2,6,6a,7,11a,11b-hexahydro-7,7-dimethyl-9*H*-pyrrolo[1’,2’:2,3]isoindolo[4,5,6-*cd*]indol-9-one (**12-96**), is a toxic metabolite which occasionally accompanies aflatoxins in contaminated materials. Its major producers are some strains of *Aspergillus flavus* and *A. versicolor*, but it is also produced by some other fungi, especially *Penicillium commune*, *P. griseofulvum* and occasionally *P. camembertii*. The presence of this mycotoxin has been demonstrated, for example, in maize (highest levels found were close to 10 mg/kg), sunflower seeds, peanuts, but also in meat products with edible moulds on casings and cheeses that have moulds on the rind or throughout, which may indicate that the strict criteria for selecting non-toxinogenic strains of moulds were not met. Some papers in the literature document frequent occurrence of cyclopiazonic acid in animal feed (hay and silage), but information about the deposition of this mycotoxin in animal tissues is not available. Generally, the data on the occurrence of cyclopiazonic acid are very limited, as well as information about its stability in the contaminated materials. Japanese studies have reported a significant decrease of the level of this mycotoxin in the production of soy sauce.

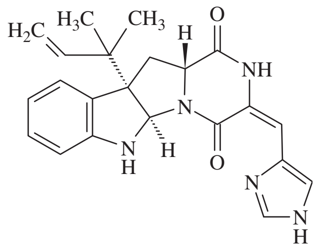


12-96, cyclopiazonic acid

The adverse health effects of cyclopiazonic acid manifest at higher concentrations. It is a specific inhibitor of calcium-ATPase, which transfers calcium after a muscle has contracted. In animals it causes necrosis of the liver and necrotic changes in the gastrointestinal tract and muscles. It is considered a potential carcinogen, but exposure limits are not yet defined.

Roquefortine C

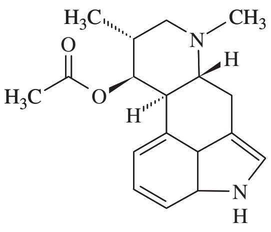
Roquefortine C, or by chemical name 10b-(1,1-dimethylprop-2-en-1-yl)-6,10b,11,11a-tetrahydro-3-(1*H*-imidazol-4-ylmethylene)-2*H*-pyrazino(1’,2’:1,5)pyrrolo(2,3-*b*)indole-1,4(3*H*,5a*H*)-dione (**12-97**), is a basic compound, which can be categorised among alkaloids. The basis of its structure is a dihydroindole skeleton. Roquefortine C can produce some strains of the fungus *Penicillium roquefortii* used to make Roquefort cheeses (blue cheeses). These fungi are commonly found in nature and were discovered by cheese makers when aging cheeses in damp, cool caves. *P. roquefortii* comprises three accepted species: *P. carneum*, which is associated with meat, cheese and bread; *P. paneum*, associated primarily with bread and silage; and *P. roqueforti*, which is associated with various processed foods and silage.



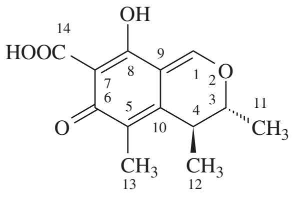
12-97, roquefortine C

At the time of its discovery, concentrations of roquefortine C in cheeses reached as much as units of mg/kg, but today the cultural strains used either do not produce this mycotoxin at all or produce it only in trace amounts. The toxicity of roquefortine is generally low, but more detailed data on its effect on the human organism are not available.

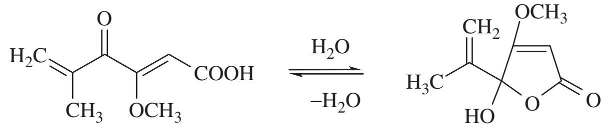
Roquefortine C was often accompanied by a structurally related mycotoxin isofumigaclavine A (**12-98**). *P. roqueforti* molds may occasionally produce patulin, citrinin (**12-99**), penicillic acid (**12-100**), the so-called PR-toxin (**12-101**) and certain other toxins that have been implicated in incidents of mycotoxicoses. However, PR toxin is not stable in cheese and breaks down to the less toxic PR imine (**12-101**). Other secondary metabolites of *P. roqueforti* found in blue cheeses are andrastins A–D with skeletons of *ent*-5α,14β-androstane. In European blue cheeses, the content of andrastin A (**12-102**) ranged from 0.1 to 3.7 mg/kg and contents of andrastins B, C and D were on average five times lower. The most significant biological activity of adrastins is the ability to inhibit the enzyme farnesyltransferase, an enzyme that catalyses the transfer of farnesyl residue from farnesyl diphosphate to proteins. It is a part of the apparatus carrying post-translational modification of proteins in the cell. Attachment of farnesyl residue to a protein changes its physico-chemical and biological effects. Such modified proteins are able to cross the lipophilic membranes and many play a role as cell signalling molecules. They have also anticarcinogenic properties.



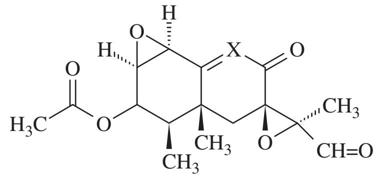
12-98, isofumigaclavine A



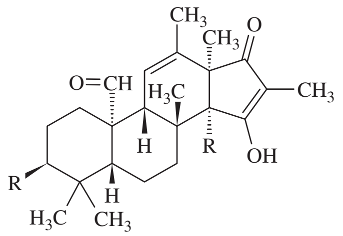
12-99, citrinin



12-100, penicillic acid



12-101, PR toxin, X = CH
PR toxin imine, X = N



12-102, adrastin A, R = $COOCH_3$

Citrinin

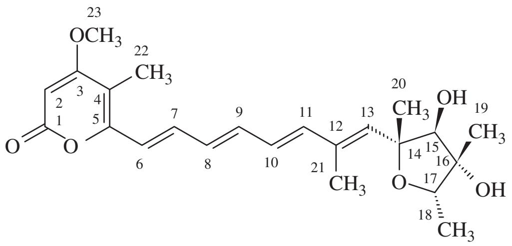
Citrinin, also known as antimycin, or by its chemical name (3*R*,4*S*)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3*H*-isochromene-7-carboxylic acid (**12-99**) can exist in two tautomeric forms. The quinoid form is normally present in neutral media and the tautomeric phenol form occurs in alkaline media. Citrinin is a secondary metabolite of fungi of the genera *Aspergillus* and *Penicillium*, especially of fungi *P. citrinum* and *P. verrucosum*. Citrinin is the main contaminant of so-called yellow rice (see Section 12.3.1). In temperate climates it is found mainly in cereals, but data concerning its occurrence are limited. Citrinin has also been found in cheeses, sake and commercial red yeast rice supplements (see Section 9.7).

Shortly after its discovery in the early 1930s, citrinin was first considered to be an antibiotic, but later its strong nephrotoxicity was

demonstrated, as well as its possible teratogenicity and interference with metabolic processes leading to damage in the liver. The intoxication of livestock (especially pigs, but also other monogastric animals) is manifested by diarrhoea and reduced weight gain. Toxic effects in humans have not been clearly documented.

Citreoviridin

Citreoviridin, with the chemical name (1*E*,3*E*,5*E*,7*E*)-6-[8-(3,4-dihydroxy-2,4-dimethyloxolan-2-yl)-7-methylocta-1,3,5,7-tetraen-1-yl]-4-methoxy-5-methyl-2*H*-pyran-2-one (**12-103**) is a typical secondary metabolite of the fungus *Penicillium citreoviride*. Citreoviridin was identified in crops grown in Asia, especially in the so-called yellow rice (see Section 12.3.1); its occurrence has been reported even in pecans and maize that had not been harvested at the time of full maturity. Information on the presence of this substance in the diet of the European population is not available, nevertheless some findings, particularly in maize, have been reported.



12-103, citreoviridin

Citreoviridin is a potent inhibitor of soluble mitochondrial ATPase. In experimental animals it has caused a disease called acute cardiac beriberi, whose symptoms are similar to the symptoms of classic beriberi (convulsions and paralysis), but the disease is not curable with thiamine.

Penicillic acid

Penicillic acid, (*Z*)-3-methoxy-5-methyl-4-oxohexa-2,5-dienoic acid and the lactone of its hydrate (5-hydroxyisoprop-5-en-1-yl-4-methoxyfuran-2-one), respectively (**12-100**), is produced by a wide range of fungi. An example is the fungus *Penicillium aurantiogrisseum* found in maize. The presence of this mycotoxin has also been documented in some sausages and on the surface of hard cheeses. Occurrence of moulds in the deeper layers below the surface is limited by the availability of oxygen.

Penicillic acid is a cytotoxic agent with antibacterial and antiviral effects. During repeated administration to experimental animals its hepatotoxicity and nephrotoxicity have been demonstrated. Penicillic acid is a relatively unstable compound, which reacts readily with sulfhydryl substances under opening the lactone ring (as patulin) and a loss of toxicity.

Trichothecenes

Trichothecenes are one of the most important groups of mycotoxins. Their main producers are microscopic filamentous fungi of the genus *Fusarium* (Table 12.21). Unlike other toxinogenic fungi, in which saprophytic species prevail, *Fusarium* species are primarily parasitic fungi, plant pathogens, and some are opportunistic infectious agents of humans and animals. The production of trichothecenes has been demonstrated even in some strains of fungi of the genus *Myrothecium* (such as *M. verrucaria* and *M. roridum*), *Trichoderma*, *Cephalosporium*, *Verticimonosporium* and *Stachybotrys*. *Stachybotrys atra* moulds were known in ancient times and are now attracting attention again. Representatives of this genus grow preferentially on substrates rich in cellulose, such as wood, paper and cotton. An unusual feature, compared with other moulds, is the high content of trichothecenes, especially T-2 toxin, in their spores. Inhalation of spores can cause pulmonary haemorrhage, and some fatalities have been reported in cases involving children.

As regards their chemical structure, trichothecenes constitute a diverse group of compounds derived from tricyclic sesquiterpenes. The most important structural features causing their biological activities are six-membered rings containing a double bond between carbons C-9 and C-10, an epoxy ring between C-12 and C-13 and hydroxyl or acetyl groups at appropriate positions on the trichothecene nucleus and the structure and position of the side chain. All trichothecenes have the same stereochemistry: α at C-3, C-7 and C-8 and β at C-4 and C-5 in A and B trichothecene types. For example, the systematic chemical name of deoxynivalenol (vomitoxin) is: (3α,7α)-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one and that of T-2 toxin is: (2α,3α,4β,8α)-4,15-bis(acetyloxy)-3-hydroxy-12,13-epoxytrichothec-9-en-8-yl 3-methylbutanoate. According to the characteristic structures related to the physico-chemical and biochemical properties, trichothecenes can be divided into four types:[5]

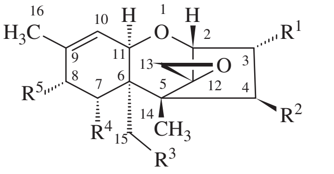
- type A trichothecenes (**12-104**) that do not have the C-8 oxo group; such as the T-2 toxin, T-2 tetraol, HT-2 toxin, diacetoxyscirpenol, neosolaniol and some other trichothecenes produced by *F. acuminatum*, *F. sporotrichiodes*, *F. sporotrichiodes* var. *tricinctum* and *F. poae*;

- type B trichothecenes (**12-105**) typically having the C-8 carbonyl group; they include, for example, nivalenol, deoxynivalenol (also called vomitoxin), 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, trichothecin and fusarenone-X produced by *F. nivale* and *F. episphaeria*;

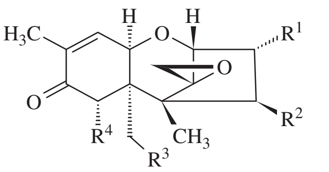
- type C trichothecenes typically have a second epoxide group at C-7/C-8 (or at C-9/C-10), such as crotocin (**12-106**), and are produced by *Cephalosporium crotocingigenum*;

- type D trichothecenes contain a macrocyclic ring between C-4 and C-15 with two ester linkages, which include, for example, verrucarin A (**12-107**).

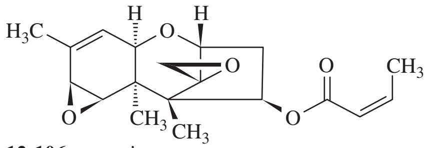
[5]Recognised may be two additional trichothecene types. The type E trichothecenes include mycotoxins with an opened macrocyclic ring and the type F trichothecenes do not have the 12,13-epoxide ring as it is replaced by a vinyl linkage with the oxygen atom in the epoxide ring removed.



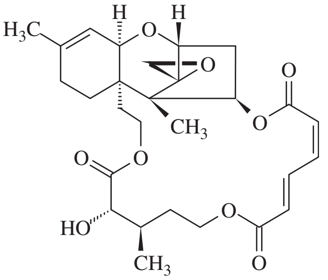
12-104, T-2 toxin, $R^1 = OH$, $R^2 = R^3 = OCOCH_3$, $R^4 = H$, $R^5 = COCH_2CH(CH_3)_2$
T-2 tetraol, $R^1 = R^2 = R^3 = R^5 = OH$, $R^4 = H$
HT-2 toxin, $R^1 = R^2 = OH$, $R^3 = OCOCH_3$, $R^4 = H$, $R^5 = COCH_2CH(CH_3)_2$
diacetoxyscirpenol, $R^1 = OH$, $R^2 = R^3 = OCOCH_3$, $R^4 = R^5 = H$
neosolaniol, $R^1 = OH$, $R^2 = R^3 = OCOCH_3$, $R^4 = H$, $R^5 = OH$



12-105, deoxynivalenol, $R^1 = R^3 = R^4 = OH$, $R^2 = H$
nivalenol, $R^1 = R^2 = R^3 = R^4 = OH$
trichothecin, $R^1 = R^3 = R^4 = H$, $R^2 = OCOCH{=}CHCH_3$
fusarenone-X, $R^1 = R^2 = R^3 = OH$, $R^4 = OCOCH_3$



12-106, crotocin



12-107, verrucarin A

Nearly 180 trichothecenes and their derivatives are currently known. While trichothecenes of types C and D do not commonly occur in crops, trichothecenes of types A and B are widespread. The presence of one and sometimes more trichothecenes has been demonstrated mainly in cereals (wheat, barley, oats and maize) grown in the temperate zones of Europe, America and Asia. Grains attacked by *Fusarium* spp. are characterised by a typical reddish colour. Findings of trichothecenes A and B have also been reported in soybeans, oil seeds, banana and mango.

One of the most trailed trichothecenes is a type B trichothecene deoxynivalenol, which is mainly produced by the fungus *Gibberella zeae*, anamorph (the asexual reproductive form in the life cycle) of *Fusarium graminearum* found mainly in warmer geographic regions (its optimum growth temperature is 25 °C). The producer is also *F. culmorum* requiring a lower temperature (its optimum is 21 °C). These microscopic fungi cause an important disease in wheat, barley, maize and other cereals called *Fusarium* head blight (FHB) that causes losses in grain yield and quality. Under conditions favourable for the growth of toxinogenic fungi, the deoxynivalenol concentration can reach up to 10 mg/kg; however, typical findings are significantly lower. The incidence of deoxynivalenol (as well as other mycotoxins) in cereals is highly variable, depending on the climatic conditions in the locality and the type of crop and, of course, the resistance of crop varieties.[6] In some years, this mycotoxin can be present in virtually 100% of examined samples.

In recent years the need for more information about the type A trichothecenes has been emphasised, not only with regard to their toxicity (this is greater than that of deoxynivalenol), but also due to the increasing trend of incidence in European cereals. Mycotoxins T-2 and HT-2 (a product of T-2 deacetylation) are found mainly in oats, where their amounts may be up to 1000 μg/kg, but the annual variability of contamination is considerable. The only way to reduce the level of type A trichothecenes in foods and feeds containing oats is the removal of bran from grains, as bran may contain 75–90% of the total amount of these mycotoxins.

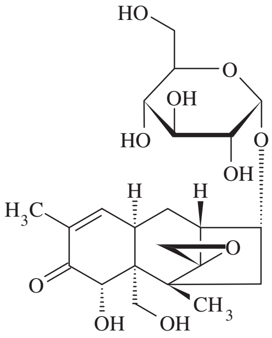
Physico-chemical properties of trichothecenes are dependent on the presence of polar substituents, therefore type B trichothecenes are generally more polar than type A trichothecenes. Under normal conditions of technological and culinary processing of cereals, trichothecenes are relatively stable toxins and pass from contaminated raw materials into the final products, such as bakery products, breakfast cereals, beer and others. During the processing of wheat in mills, a significant portion of these mycotoxins (up to 50%) is removed, but their contamination in the bran is roughly two to three times higher than the original contamination in the grain. Wholewheat flour derived by grinding the whole grain generally contains higher amounts of trichothecenes than white flour, as its content of mycotoxins depends on the degree of milling (four extraction rate) and is generally 20–70% of the original amount in the grain. For example, flour produced from wheat with a deoxynivalenol content of 0.51 mg/kg contained 0.35 mg/kg of this mycotoxin, while its concentration in the bran increased to 1.12 mg/kg.

The results of studies focused on changes in the content of trichothecenes in the production of bread and other bakery products are sometimes very different, nevertheless some decrease in contamination of flour was observed, especially in fermented products, where the losses of deoxynivalenol ranged from 15 to 56%. A decrease in concentrations of deoxynivalenol (up to 40%) and some other mycotoxins (nivalenol and acetyloxynivalenols) also occurs after the addition of sulfites to the dough, as sulfites react with trichothecene mycotoxins with the addition to the C-9/C-10 double bond yielding the corresponding hydroxysulfonates. A significant reduction in the content of trichothecenes, especially

[6]The resistance of cereals is classified into different groups: type I is resistance to primary infection, type II resistance to the spread of infection, type III is the resistance to infection of grains, type IV is tolerance against *Fusarium* head blight (FHB) and trichothecenes, type V is the resistance to the accumulation of trichothecenes: Class 1 is chemical modification (such as conjugation), Class 2 is inhibition of biosynthesis.

B group mycotoxins, is also achieved by washing the contaminated grains, as mycotoxins mainly occur on the surface of grains. Such a reduction in the mycotoxin content occurs during barley steeping, the purpose of which is to evenly hydrate the endosperm mass of barley, raising the moisture level to allow uniform growth during its germination. For example, barley with extremely high deoxynivalenol concentration of 16.1 mg/kg contained only 8% of this amount after 24 h of steeping. In aqueous solutions, deoxynivalenol is relatively stable and is not decomposed during brewing. Sometimes a slight increase in its concentration compared with the concentration in malt can even be recorded.

Studies in recent years have shown a more serious aspect of trichothecene mycotoxins, which should be considered when assessing exposure risks. In addition to their free, routinely monitored forms, plant materials contain various conjugates, which are either water soluble (so-called masked mycotoxins) or insoluble (so-called bound mycotoxins). In the former case, the conjugates with mono- or oligosaccharides arise during the detoxification process in contaminated plants as other conjugates of xenobiotics. Until recently, masked mycotoxins escaped routine analysis, due to their different physico-chemical properties (especially higher polarity). The best known conjugated trichothecene is deoxynivalenol-3-β-D-glucoside (**12-108**), which may occur, for example in wheat, in amounts corresponding to up to 30% of the molar concentration of free deoxynivalenol. Relatively high levels of deoxynivalenol-3-β-D-glucoside were recently found in malt, beer and other fermented cereal products, when the molar ratio of deoxynivalenol-3-β-D-glucoside to deoxynivalenol in some samples may be even ≥1. Increased concentrations of deoxynivalenol, deoxynivalenol-3-β-D-glucoside and other fusariotoxins during the production of malt and beer can be an order of magnitude higher, which is probably related to the activity of hydrolytic enzymes that contribute to the release of these mycotoxins from their linkages to polysaccharides such as starch. Increased levels of mycotoxins may be also due to *de novo* activity of potentially present moulds. Data on the bioavailability and toxicity of deoxynivalenol-3-β-D-glucoside are not yet available, but previous research suggests that enzymes of intestinal bacteria are able to cleave this conjugate, which may lead to additional release of deoxynivalenol in mammals. It would however mean that the currently evaluated dietary exposure to deoxynivalenol is underestimated.



12-108, deoxynivalenol-3-β-D-glucopyranoside

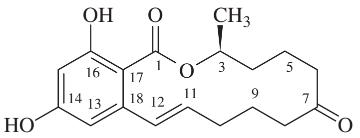
Foods of animal origin do not contribute significantly to the exposure of consumers, as the transfer of trichothecenes into meat, milk and eggs is insignificant because trichothecenes undergo rapid detoxification (epoxide ring opening and formation of glucuronides) after absorption in the digestive tract. In livestock (including cattle and pigs), acetylated trichothecenes are easily hydrolysed by enzymes of the intestinal microflora.

Trichothecenes exhibit a wide range of biological effects, such as antibacterial, antiviral, cytostatic and fungistatic; some of them are also phytotoxic. Toxicity to vertebrates varies over a considerable range. Based on experiments with animals, some of them (especially T-2 toxin as a representative of the type A trichothecenes) are mutagenic and immunotoxic (immunosuppressive) compounds that lower the body's normal immune response. In humans, exposure to T-2 toxin is associated with a disease called alimentary toxic aleukia (ATA), which is manifested by a marked reduction or the complete absence of leukocytes or platelets in blood serum. The disease has been reported in the temperate zone, which stretches from the south of Russian Siberia to the Balkans. The largest epidemic was recorded in the former Soviet Union in the 1940s, when, as a result of the war, unharvested grains (mainly millet) in fields were covered with snow and were attacked by *Fusarium* moulds. The grain harvested the following spring was extremely contaminated and caused the deaths of more than 17 000 people. To a lesser extent, alimentary toxic aleukia occurred in the 1950s and 1960s in Hungary and France. Typical symptoms of intoxication are gastrointestinal inflammation, vomiting and diarrhea, often with headaches. In later stages, the intoxication leads to a reduction in the number of platelets and white blood cells, in the third stage to various infections caused (in healthy people) by banal harmless microflora.

Another type A trichothecene, diacetoxyscirpenol, is significantly less acutely toxic in contrast to T-2 toxin, but is characterised by significant teratogenic potential. Deoxynivalenol, a representative of the type B trichothecenes, is the least toxic mycotoxin of this group, but is often the main trichothecene in contaminated foods. Signs of acute intoxication caused by deoxynivalenol are vomiting, abdominal pain, diarrhea and headaches associated with dizziness. Extremely high toxicity is shown by macrocyclic trichothecenes of type D. For example, the acute toxicity of verrucarin is still about ten times higher than that of T-2 toxin. In comparison with aflatoxins, trichothecenes exhibit direct biological effects without metabolic activation. It is expected that they react with some cellular components, such as ribosomes, which results in inhibition of protein synthesis. The IARC, however, has not included trichothecenes among human carcinogens. Currently, maximum limits are set only for deoxynivalenol (Table 12.23).

Zearalenone

Zearalenone, also known as mycotoxin F_2, or (3*S*,11*E*)-14,16-dihydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione (**12-109**), is another toxicologically significant secondary metabolite of filamentous fungi of the genus *Fusarium*. The major producers of zearalenone are fungi *F. culmorum*, *F. graminearum*, *F. cerealis*, *F. equiseti* and *F. semitectum*. Zearalenone is found mostly in cereals, especially in maize, but often also in wheat, barley and oats and sometimes in



12-109, zearalenone, (*E*)-isomer

spices. Producers of zearalenone are found in virtually all climatic zones, and almost without exception the positive samples (typical concentrations are in the range of tens to hundreds of μg/kg) are also contaminated by fusariotoxins (deoxynivalenol and nivalenol) and by fumonisins. Some *Fusarium* strains also produce other zearalenone derivatives, whose concentrations can be 10–20% of the free mycotoxin. For example, (*E*)-zearalenone-related metabolites from cultures of *F. graminearum* are 13-formylzearalenone, 5,6-dehydrozearalenone, two epimers of 5-hydroxyzearalenone and 10-hydroxyzearalenone, α- and β-epimers of zearalenol, α- and β-zearalanol (*Z*)-zearalenone and (*Z*)-zearalenol.

The dominant secondary metabolite found in maize, but also in wheat, is zearalenone-14-β-D-glucoside, which is accompanied by the corresponding malonate and diglycosides (diglucoside and xylosylglucoside). Fungi *Thamnidium elegans* also produce zearalenone 14,16-diglucoside. Another metabolite identified only in maize is zearalenone-14-sulfate. The formation of zearalenone conjugates was studied intensively in the model plant thale cress (*Arabidopsis thaliana*), which led to identification of glucoside, malonylglucoside, diglucoside, triglucoside, xylosylglucoside and α-zearalenol and β-zearalenol. It is assumed that these recognised metabolites may also be present in cereals (Figure 12.43).

Zearalenone is relatively thermostable and does not decompose, for example, even when heated to $120\,^{\circ}\mathrm{C}$ for 4 h; therefore the loss is mainly attributed to the formation of complexes with components of the material. Irradiation by UV light causes isomerisation of the natural (*E*)-isomer (*trans*-isomer) to (*Z*)-isomer (*cis*-isomer), (3*S*,11*Z*)-14,16-dihydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione (**12-110**).

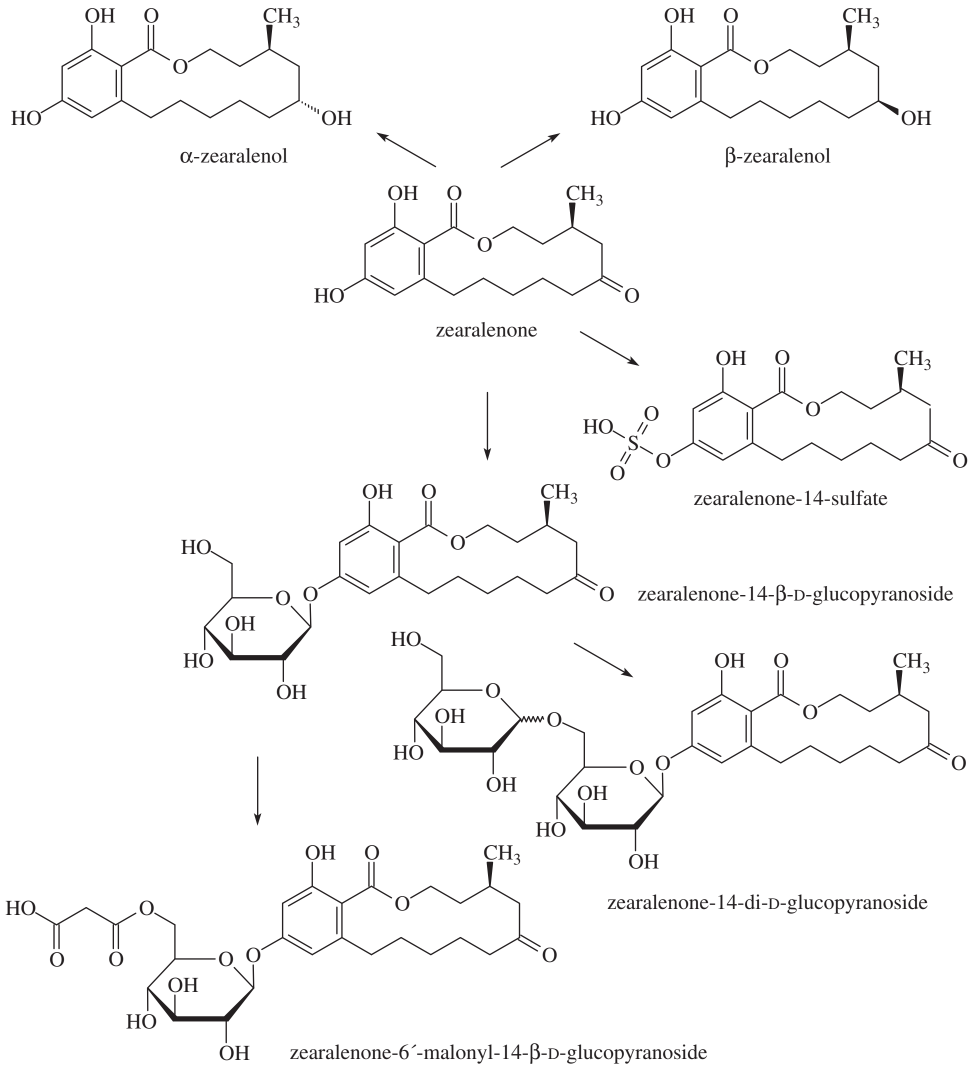
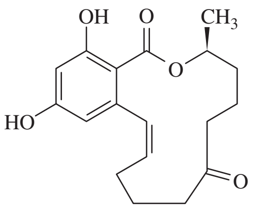


Figure 12.43 Zearalenone derivatives in plants.



12-110, zearalenone, (*Z*)-isomer

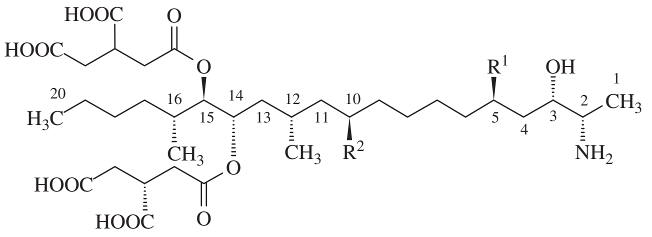
Washing of maize, which is effective for decreasing the concentration of deoxynivalenol and other trichothecenes, does not lead to a significant decrease in the amount of zearalenone, probably due to its hydrophobicity. As a relatively stable compound, zearalenone is transferred to cereal products, including bread, malt and beer. Zearalenone is relatively lipophilic, therefore is also found in vegetable oils, especially in germ oils. As in the case of other fusariotoxins, the content of zearalenone in flour is lower than that in the original grains and depends on flour extraction rate. For example, white flours made from contaminated wheat contain only 30–50% of the initial amount of zearalenone, while zearalenone concentrations in bread (compared with its content in flour) decrease by 34–40%. Other experiments have shown the residual content of zearalenone was 60% of the initial amount in bread and 80% in biscuits. In the production of pasta in the presence of 1% potassium carbonate, the loss of zearalenone was 48–62%.

Zearalenone is rapidly metabolised in the liver of humans and animals. Primarily, the C-7 oxo group is reduced to give α- and β-zearalenol and the C-11 double bond is reduced to yield the corresponding zearalanols (see Section 12.8.3.1). These products are then secreted as soluble glucuronides, but in small amounts can be found in milk and eggs, but this route of human dietary exposure is marginal. Acute toxicity of zearalenone is low; however, zearalenone and its derivatives exhibit significant oestrogenic and anabolic effects. Dietary intake may cause (thanks to the structural similarity with the steroid hormones oestrogens) hyper oestrogenic syndrome. Oestrogenic potential is approximately one tenth of the potential of 3,17β-estradiol. Generally, the binding affinity to the oestrogenic receptors decreases as follows: α-zearalanol > α-zearalenol > β-zearalanol > β-zearalenol. With regard to the oestrogenic effect, zearalenone is sometimes referred to as a mycoestrogen (see Section 10.3.3.5.1). The IARC ranks zearalenone in Category 3 (compounds unclassifiable as to carcinogenicity in humans), but maximum limits are set for selected cereal commodities (Table 12.23).

Fumonisins

This group of mycotoxins was only discovered in the late 1980s in South Africa. Twenty filamentous fungi of the genus *Fusarium* have been idenitified, which biosynthesise these secondary metabolites, however, the main producers of fumonisins are fungi *F. moniliformis* and *F. proliferatum*. Fumonisins are found in cereals, most notably in maize (where their contents can reach tens of mg/kg) and maize products, such as maize flakes, polenta and other products. The presence of fumonisins was also demonstrated in rice, millet and other cereals. Findings in animal feed, especially in silage, are also common.

Dozens of secondary metabolites from the fumonisin group are currently known, which are divided, according to their structure, into four groups (A, B, C and P) that mainly include relatively polar diesters of propane-1,2,3-tricarboxylic acid with 2-amino- or 2-acetylamino-12,16-dimethyl-3,5,10,14,15-pentahydroxyeicosane (**12-111**). In terms of toxicity or chemical food safety, monitored mycotoxins are B group fumonisins. The most common compounds occurring in the highest concentration are fumonisins B_1 and B_2, which often occur in a ratio of about 3 : 1. Fumonisin B_3 can often be found in materials with higher levels of contamination. For example, the systematic chemical name of fumonisin B_1, also known as macrofusine, is (2*S*,2′*S*)-2,2′-{[(5*S*,6*R*,7*R*,9*R*,11*S*,16*R*,18*S*,19*S*)-19-amino-11,16,18-trihydroxy-5,9-dimethylicosane-6,7-diyl]bis[oxy(2-oxoethane-2,1-diyl)]}disuccinic acid. Fumonisin B_1, the predominant metabolite, is known to cause a range of species-specific toxic responses, including leucoencephalomalacia in horses, pulmonary oedema in swine and hepatosis and nephrotoxicity in rodents. It is known to intervene in the sphingolipid metabolism by inhibition of ceramide synthase, which is believed to be the key event in the toxicogenesis of fumonisins.



12-111, fumonisin B_1, $R^1 = R^2 = OH$
fumonisin B_2, $R^1 = OH$, $R^2 = H$
fumonisin B_3, $R^1 = H$, $R^2 = OH$

Like many other mycotoxins, fumonisins are relatively stable even at higher temperatures. For example, a significant decrease in the concentration of fumonisins in maize meal occurs during heating to 200 °C for 60 min. During baking of muffins from contaminated maize flour at 220 °C, a noticeable reduction of the amount of fumunisins B_1 and B_2 occurred after about 30 min of baking. With normal thermosterilation of sweet maize, losses of fumonisins were around 15%, rarely more. Higher pH values may cause hydrolysis of one or both propane-1,2,3-tricarboxylic acids. This reaction proceeds, for example, during the production of some speciality maize products, such as authentic Mexican *tortillas* and *nachos* prepared from maize flour with the addition of lime milk (see Section 5.8.5.2), and results in a reduction of fumonisin B_1 content of up to 60% of the original amount. Hydrolysed fumonisins may occur in products at a level of up to 20% of the parent mycotoxin amount. Their toxicity has not yet been evaluated, but it is suggested that they may be more toxic than the parent compounds, because they are less polar and therefore more easily absorbed by the intestinal mucosa.

Losses of fumonisins during technological processing of contaminated materials may be due to reactions with some components of the material, which yields bound forms of mycotoxins. Reaction depends mainly on the composition of the raw materials and the conditions employed during processing. Figure 12.44 illustrates the formation of conjugates of fumonisin B_1 with starch and proteins, which takes place at higher temperature. The first reaction step is dehydration to fumonisin bisanhydride that subsequently reacts with either protein (via lysine ε-amino groups and sulfhydryl groups of bound cysteine) or starch. In the reaction of fumonisin B_1 with reducing sugars, the amino group of the side chain enters the reaction, which yields analogous products which also arise in the Maillard reaction. In model experiments and real foods, the corresponding glycosylamines, Amadori products and also other products are produced. The identified adducts from reaction of fumonisin B_1 with glucose include *N*-(1-deoxy-D-fructos-1-yl)fumonisin B_1, *N*-methylfumonisin B_1, *N*-(carboxymethyl)fumonisin B_1, *N*-(3-hydroxyacetonyl)-fumonisin B_1, *N*-(2-hydroxy-2-carboxyethyl)fumonisin B_1 and *N*-methylfumonisin B_1.

Toxicity of fumonisins is caused by inhibition of the enzyme sphingosine *N*-acetyltransferase (ceramide synthase) due to the structural similarity of fumonisins with sphingosine (see Section 3.5.1.1.3). The toxic effects are closely related to the presence of the amino group in the molecule of fumonisin; the reaction products with reducing sugars are virtually non-toxic as the amino group is blocked. Fumonisin B_2 is more cytotoxic than fumonisin B_1.

The assessment of dietary risk of exposure to fumonisins is relatively complicated with regard to a variety of individual mycotoxins. In some types of maize products, fumonisins that are not currently monitored may significantly contribute to the toxic effects. Fumonisins cause a number of livestock diseases, such as mycotoxicosis known as Equine LEucoencephaloMalasia (ELEM) in horses or Porcine Pulmonary oEdema (PPE) in pigs. Intoxication by higher doses of mycotoxins can be fatal in both cases. In connection with the possible occurrence of fumonisins in foods, their hepatotoxicity and nephrotoxicity to humans and carcinogenicity demonstrated

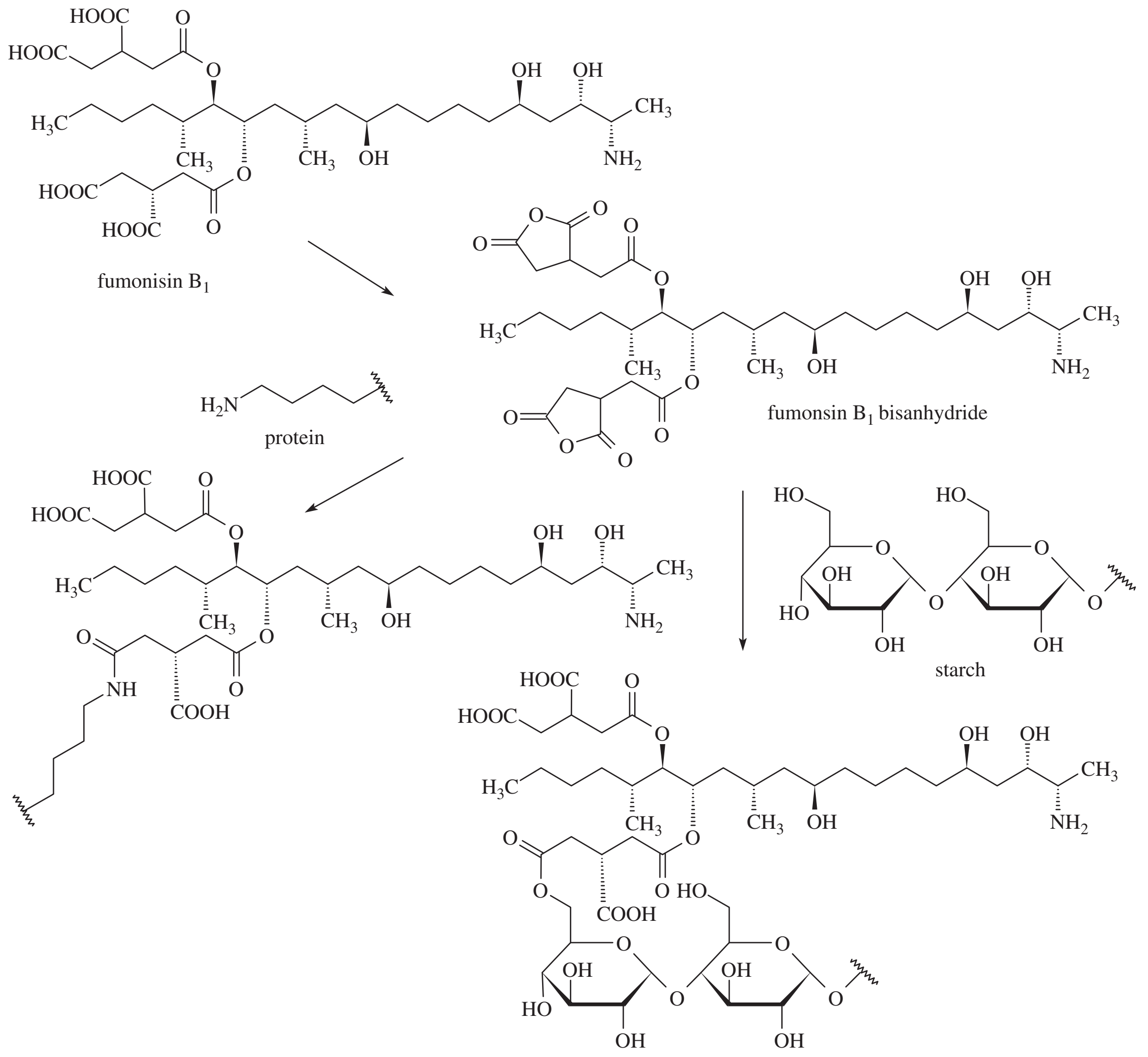
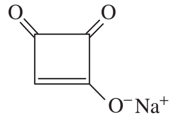


Figure 12.44 Reaction of fumonisin B_1 with proteins and starch.

in animals are often discussed. Studies from South Africa and China document a possible contribution of high doses of fumonisins from maize in the aetiology of human oesophageal cancer. According to the IARC, fumonisins are classified as class 2B carcinogens,[3] and are characterised as promoters of carcinogenic processes. For fumonisins B_1 and B_2, maximum limits are set for the sum of two major mycotoxins (Table 12.23) in selected cereal commodities. The tolerable daily intake (TDI) for fumonisins B_1, B_2 and B_3 alone or in combination is 2 μg/kg body weight.

Moniliformin

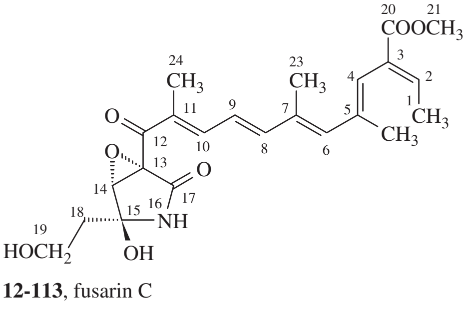
Moniliformin sodium salt (sodium 3-hydroxycyclobut-3-ene-1,2-dione, **12-112**) was originally isolated from substrates attacked by fungus *Fusarium moniliformis* (syn. *F. verticillioides*). Now five producers of this genus are known (*F. avenaceum*, *F. subglutinans*, *F. proliferatum* and others). Moniliformin is most often found in maize, but is also phytotoxic to wheat and tobacco. Poultry is very sensitive to this mycotoxin, especially chickens and ducklings. The toxicological evaluation of this toxin is not yet complete and hygienic limits have not been established.



12-112, moniliformin

Fusarin C

Fusarin C (**12-113**) is a co-metabolite of the parasitic fungus *Fusarium moniliformis* (syn. *F. verticillioides*) occurring primarily on maize, but it was also found, along with moniliformin, in other cereals. Of the nine known producers of fusarin C (*F. graminearum*, teleomorph of *G. moniliformis Gibberella fujikuroi*, *Fusarium venenatum* and others), seven have been isolated from European crops and soils. Fusarin C is a potent mutagen and a potential human carcinogen. From this perspective, its toxicity is similar to that of aflatoxin B_1 and sterigmatocystin.



12-113, fusarin C

Ergot alkaloids

Ergot fungi refer to a group of fungi of the genus *Claviceps*, which includes about 50 species occurring mostly in the tropical regions. The most prominent member of the genus *Claviceps* is the common fungus *C. purpurea*. This fungus parasitises on certain cereals, especially on rye and triticale, but also on wheat. The infested grain is transformed by the fungus into black-to-dark purple sclerotium, known as ergot. In some cereals, ergot bodies may reach up to 20 mm in length, but in forage grasses they remain small and slender. Cereal heads may contain one or several ergot bodies, which contain an interesting group of ergot alkaloids with a wide range of biological effects. Ergot alkaloids are used today in medicine, especially in neuroendocrinology and in treatment of diseases associated with impaired neural transmission in both the central and peripheral nervous systems (e.g. migraine, Parkinson's disease or senile dementia).

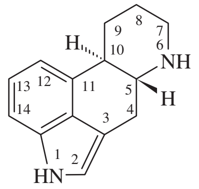
Historically, these alkaloids sometimes caused a very serious disease called ergotism, the first mycotoxicoses described in humans. Epidemics of ergotism were documented in ancient Greece and large epidemics were recorded in the Middle Ages in Europe, particularly in populations that relied on bread prepared from rye flour which was contaminated by ergot alkaloids. *Ergotism gangraenosus* was a type of poisoning that affected the circulatory system (symptoms included swelling of the limbs, their inflammation and, in the last stage, gangrene), while *ergotism convulsivus* was manifested primarily by an impaired nervous system (symptoms were hallucinations and ecstasy). The apparent randomness of these epidemics and the helplessness of people against their effects were still fertile ground for the spread of religious beliefs in the early modern period, as evidenced by the name of the diseased state: Holy Fire ('*ignis sacer*' in Latin) or St. Anthony's fire ('*ignis sancti Antonii*'). The last case of mass-poisoning in Europe was described in the French village of Pont-Saint-Esprit in 1951. Outside Europe, a milder convulsive form of ergotism with no fatalities occurred in India in 1975. The last recorded outbreak of gangrenous ergotism occurred in Ethiopia in 1977–1978, when 140 people showed signs of intoxication with high mortality (34%). In 2002, a severe outbreak of gangrenous ergotism was again reported in Ethiopia.

In the last two decades, investigations have indicated an increase in the occurrence of *Claviceps purpurea* infections. This increase seems to be associated with the more extensive use of hybrid varieties of rye and perennial rye breeds and changes of agro-technical practices, especially in years when continuous moist conditions prevail during the stages of the disease cycles. First, moisture is needed at the soil surface during spring and early summer to promote germination of ergot bodies. Second, wet, cloudy and cool weather extends the period of flowering and increases the possibility for spores to enter the florets. Favourable temperatures for their growth are in the range of 18–30 °C. Unfortunately, there is not much a farmer can do to control ergot in the field, but ergot bodies are relatively easy to clean from the seed lot, especially if a gravity separator is used.

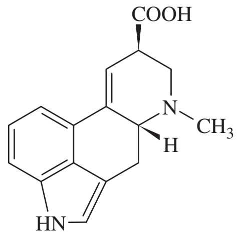
The ergot sclerotia contain from 0.15 to 0.5% alkaloids synthesised by a combination of fungal and plant metabolisms and more than 50 have been characterised. Ergot alkaloids are 3,4-disubstituted indole derivatives derived from the tetracyclic ring of ergoline, (6a*R*)-4,6,6a,7,8,9,10,10a-octahydroindolo[4,3-*fg*]quinoline (**12-114**), which has a C-9 double bond, a methyl group at N-6 and is substituted at C-8, therefore ergot alkaloids are in fact derivatives of lysergic acid, also known as (+)-D-lysergic acid

(**12-115**) and by chemical name 7-methyl-4,6,6*a*,7,8,9-hexahydroindolo[4,3-*fg*] quinoline-9-carboxylic acid, or 6-methylergol-9-ene-8-carboxylic acid, which is typically bound as an amide with an amino alcohol as in ergometrine (**12-116**) or with a small polypeptide structure as in ergotamine (**12-117**). The building blocks for lysergic acid are tryptamine and an isoprene unit. The relative representation of individual alkaloids is different in various producer strains and also differs depending on the host plant and of environmental factors. Ergot alkaloids are accompanied by simpler bases, biogenic amines (such as histamine and tyramine) and betaines (2-mercaptohistidine known as ergothioneine, **2-56**). Ergot alkaloids can be divided according to the structure into three main groups:

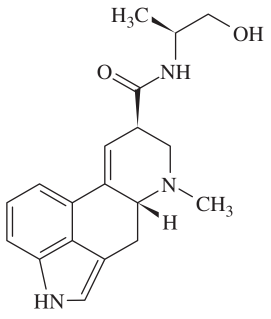
- clavines (hydroxy- or dehydroderivatives of 6,8-dimethylergoline)
- simple lysergic acid derivatives (typically amides)
- peptide ergot alkaloids known as ergopeptines (peptides derived from lysergic acid).



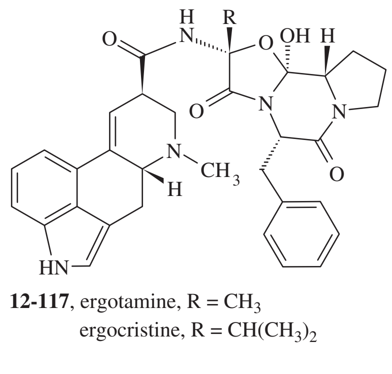
12-114, ergoline



12-115, lysergic acid

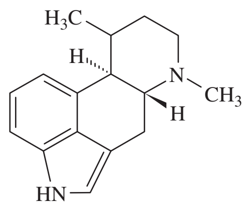


12-116, ergometrine

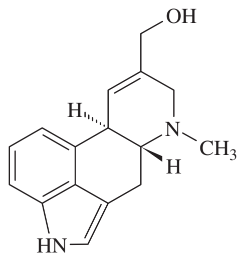


12-117, ergotamine, R = CH_3
ergocristine, R = $CH(CH_3)_2$

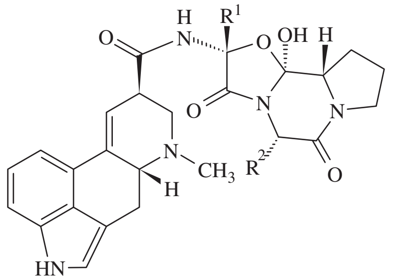
Clavines (clavine alkaloids), such as agroclavine (**12-118**) and elymoclavine (**12-119**) are considered precursors to other ergot alkaloids. At the end of the 1930s, a hallucinogenic drug – lysergic acid diethylamide (LSD) – was synthesised by modification of natural derivatives of lysergic acid. In terms of chemical food safety, the European Food Safety Authority (EFSA) has identified ergometrine (**12-116**), ergotamine, ergocristine (**12-117**), ergocornine, ergosine and ergocryptine (α-ergocryptine) (**12-120**) as the most important mycotoxins.



12-118, agroclavine



12-119, elymoclavine



12-120, ergocornine, $R^1 = R^2 = CH(CH_3)_2$
ergosine, $R^1 = CH(CH_3)CH_2CH_3$, $R^2 = CH_3$
ergocriptine, $R^1 = CH(CH_3)_2$, $R^2 = CH(CH_3)CH_2CH_3$

During processing of contaminated grains, alkaloids from the sclerotium may pass into flour and then into the final products, but concentrations of ergot alkaloids in contaminated flours rarely exceed 0.1 mg/kg. Rye flours may commonly contain higher amounts of alkaloids than wheat flours. For example, a monitoring of Swiss flours in 1985 found that the total alkaloid content in rye flours was 0.015–0.397 mg/kg and in white and brown wheat flours 0.004 and 0.103 mg/kg, respectively. Monitoring in Sweden in 1993 found the highest concentration in wheat and rye products to be 0.024 mg/kg, with ergotamine as the most frequently detected toxin, while ergocristine was the major alkaloid detected in wheat and rye flour at levels up to 0.062 mg/kg in Canada in 1980.

With the exception of ergometrine, ergot alkaloids are relatively nonpolar compounds and therefore are not very soluble in water. Lysergic acid is a chiral compound with two stereo centres. The isomer with inverted configuration at C-8 close to the carboxy group is called D-isolysergic acid. Inversion at C-5 close to the nitrogen atom leads to L-lysergic acid and L-isolysergic acid, respectively. Alkaloids with a double bond between carbons C-9 and C-10 (ergolenes) are susceptible to epimerisation, especially in non-acidic media (Figure 12.45). The laevo-rotating ergopeptins with configuration (*R*) at C-8 thus give rise to dextro-rotating (*S*)-isomers

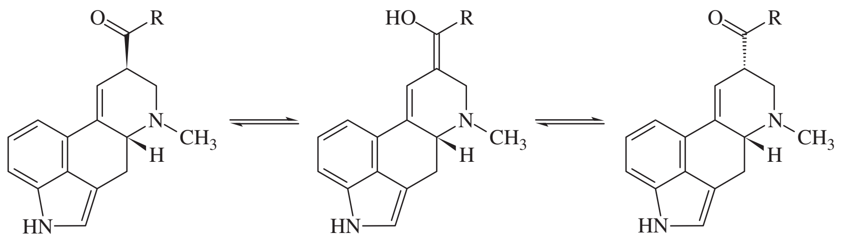


Figure 12.45 Isomerisation (epimerisation) of ergot alkaloids.

(D-isolysergic acid derivatives) that are called ergopeptinins. The individual isomers differ in both biological and physico-chemical properties. Their amount increases with unsuitable conditions of grain storage.

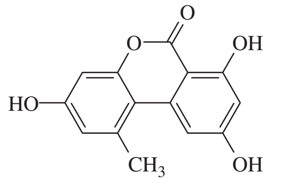
In addition to ergot, ergot alkaloids occur in some higher plants. For example, derivatives of D-lysergic and D-isolysergic acids were isolated from the seeds of beach moonflower (*Ipomoea violacea*) and the related Christmas vine *Turbina corymbosa* (Convolvulaceae). The seeds of beach moonflower were used traditionally among Mexico's Zapotec Indians for ceremonial and curative purposes.

Currently, no country regulates ergot alkaloids in food, and only in Canada and Uruguay are ergot alkaloids regulated in feeds. For example, the Canadian guideline limits for ergots in feed for swine is 6000 μg/kg, 3000 μg/kg in feed for dairy cattle, sheep and horses and 9000 μg/kg for chicks. In the EU the issue of ergot alkaloids is currently under discussion by CONTAM, an EFSA panel, and maximum limits at a level of 400–500 μg/kg in grain intended for human consumption are being discussed. For example, in India regulatory measures exist for the occurrence of ergot bodies in cereals and the designed limit is 0.01% (1 ergot body per 10 000 grains). At present, the maximum permissible level in the United States and Canada is 300 mg ergot per kg grain. Feed materials exceeding this limit are labelled 'ergoty'.

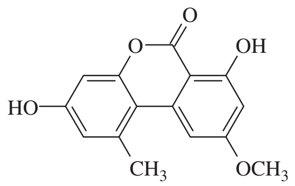
Alternaria toxins

Many species of the genus *Alternaria* can invade crops at the pre- and preharvest period and cause considerable losses due to rotting of various food crops, mainly fruits and vegetables. Owing to their ability to grow even at low temperatures, they are also responsible for spoilage of these commodities during refrigerated transport and storage. Several *Alternaria* species are known producers of toxic secondary metabolites known as *Alternaria* mycotoxins. The best-known representative of these fungi is *A. alternata* found on cereals, sunflower seeds, oilseed rape, olives, various other fruits and vegetables, especially on tomatoes.

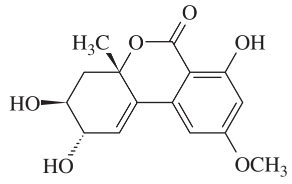
Alternaria mycotoxins can be divided into three groups based on their structure. The first group are derivatives of dibenzopyrone, which include alternariol, 3,7,9-trihydroxy-1-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (**12-121**), isolated in 1953 as the first alternaria toxin, alternariol monomethyl ether, 3,7-dihydroxy-9-methoxy-1-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (**12-122**) and altenuene, 2α,3α,4aβ-tetrahydro-2,3,7-trihydroxy-9-methoxy-4a-methyl-6*H*-dibenzo[*b*,*d*]pyran-6-one (**12-123**). The second group includes derivatives of perylene represented by altertoxin I, 1,2,7,8,12b-pentahydro-1,4,6b,10-tetrahydroxyperylene-3,9-dione (**12-124**) and derived epoxide altertoxin II, perylo(1,2-b)oxirene-7,11-dione (**12-125**). In the third group are tetramic acid (1,5-dihydro-4-hydroxy-2*H*-pyrrole-2-one) derivatives, which include the most studied mycotoxin of this group tenuazonic acid, (5*S*,6*S*)-3-acetyl-5-*sec*-butyl-4-hydroxy-3-pyrrolin-2-one (**12-126**).



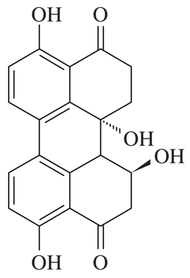
12-121, alternariol



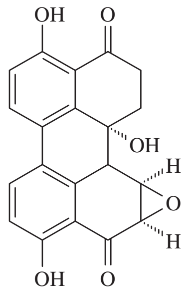
12-122, alternariol methyl ether



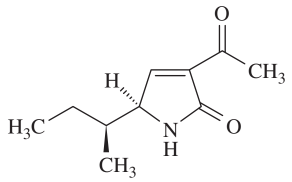
12-123, altenuene



12-124, altertoxin I



12-125, altertoxin II



12-126, tenuazonic acid

Information on the occurrence of alternaria mycotoxins in food and feed is relatively limited. For example alternariol, its methyl ether and tenuazonic acid were frequently detected in apples, apple products, apple juice concentrates, mandarins, olives, pepper, red pepper, tomatoes, tomato products, oilseed rape meal, sunflower seeds, sorghum, wheat and edible oils (olive oil, rapeseed oil, sesame oil and sunflower oil). The maximum levels reported in foods are

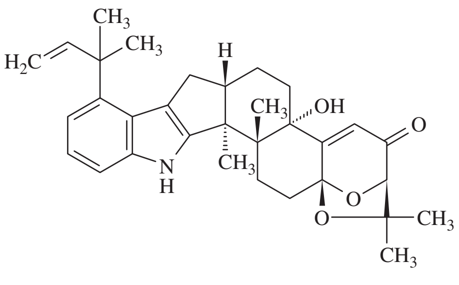
in the range of about 1–1000 μg/kg; higher levels are commonly found in visibly infected rotted products that are not suitable for consumption. Alternaria mycotoxins are relatively stable under conditions of technological and culinary food processing.

Exposure to *Alternaria* toxins has been linked to a variety of adverse health effects. They exhibit only low acute toxicity (the most toxic substance is tenuazonic acid) and their chronic toxic effects are not yet fully described. Mycotoxicoses however were described not only in animals, but have also been associated with alimentary toxic aleukia, a frequently fatal mycotoxicosis that followed the ingestion of overwintered grain or grain byproducts infested with fungi. This intoxication primarily affected poor rural families in the former USSR in the 20th century. Toxicological studies demonstrated the ability of tenuazonic acid to inhibit protein synthesis; alternariol and its methyl ether were shown to be teratogenic. Alternaria toxins are generally highly cytotoxic (for bacterial and mammalian cells) and from a toxicological point of view altertoxins are of the highest concern, probably being mutagenic, but their mutagenicity is at least one order of magnitude lower in comparison with aflatoxin B_1. Exposure limits as tolerable daily intake (TDI) or a Provisional Maximum Tolerable Daily Intake (PMTDI) for alternaria toxins have not been derived. Monitoring of foods may give impetus to further toxicological studies.

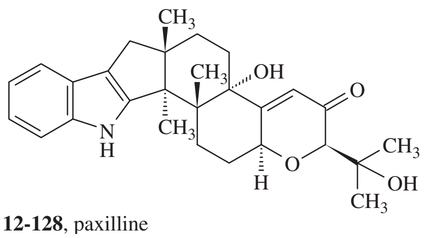
Tremorgens

Tremorgens include a rather heterogeneous group of more than 30 alkaloids (indole diterpenoids). Producers of tremorgens are fungi of the genus *Aspergillus* and also fungi of some other genera, such as *Penicillium* or *Claviceps*. The first tremorgen aflatrem, 9-(1,1-dimethylprop-2-en-1-yl)-2,3,5b,6,7,7aα,8,13,13b,13c,14,15-dodecahydro-5bβ-hydroxy-2,2,13bβ,13cα-tetramethyl-4*H*-3β,15aβ-epoxy-1-benzoxepino[6′,7′:6,7]indeno[1,2-*b*]indol-4-one (**12-127**) was isolated from the mycelium of *Aspergillus flavus* and is also produced by other *Aspergillus* species, such as *A. miniscle-rotigenes*. Another example of this group of mycotoxins is paxilline (or paxiline, **12-128**), which is produced by fungus *Penicillium paxilli*. Fungus *Claviceps paspali* produces ergot alkaloids in sclerotia and also tremorgens called paspalitrems, such as paspalitrem A (**12-129**), B and C. Paspalitrem B contains a 3-hydroxy-3-methylbut-1-(en-1-yl) unit instead of 3-methylbut-2-en-1-yl unit and paspalitrem C differes from paspalitrem A only by the position of attachment of the 3-methylbut-1-en-1-yl substituent to the indole ring.

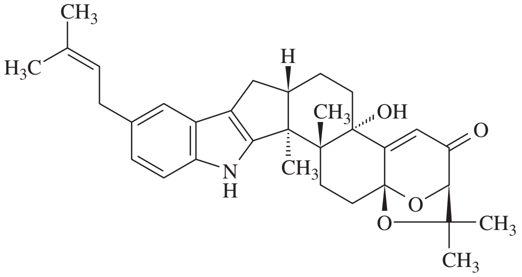
Tremorgens were found in a number of commodities, such as maize, wheat, nuts, silage and other feeds. Tremorgenic mycotoxins



12-127, aflatrem



12-128, paxilline



12-129, paspalitrem A

induce neurologic symptoms ranging from mental confusion to tremors, seizures and death, and are apparently the only class of mycotoxins with significant central nervous system activity. Tremorgens have been implicated in a number of neurologic diseases of cattle, collectively known as staggers syndromes. They pose significant agricultural and health problems for both cattle and humans. Toxicological evaluation of tremorgens is not complete and hygienic limits have not been established.

12.3.1.2.2 Human exposure

Unequivocally, the most important route of human exposure to mycotoxins are contaminated foods, although in certain circumstances a significant risk may be associated with the inhalation of mould spores or dust particles containing mycotoxins (such as when handling contaminated material). Contamination of the pharmaceutical preparations prepared by biotechnological processes, in which the filamentous fungi are used, is also possible. Exceeding of health limits for individual mycotoxins in foods in the European Union happens only in extremely rare cases. Health risks of lifelong (chronic) exposure even to very low concentrations of mycotoxins (often represented by several compounds) are essentially unknown.

12.3.1.2.3 Mitigation

Preventive measures

The optimum way to reduce the occurrence of mycotoxins in human food represents a complex of three basic preventive measures:

- reduction of infection by toxinogenic fungi in the growth period of crops;
- fast and efficient drying of harvested crops and their proper storage;

- use of fungicides to inhibit growth of fungi and fungal spores in the growth period of crops and during crop storage.

In many cases it is also necessary to address the situation when food raw materials or final food product are already contaminated with mycotoxins. Their at least partial rescue can sometimes be solved by using a variety of physical, chemical or biological methods.

Preharvest measures

Measures to minimise contamination of agricultural crops in the preharvest period are closely related to the aspects listed previously. These measures can significantly reduce the incidence of toxins produced by fungi of the genus *Fusarium* (trichothecenes and zearalenone), ergot alkaloids, tremorgens and even aflatoxins. Historically, the oldest successeful method is a reduction in the amounts of ergot alkaloids through effective application of preventive measures, such as crop varieties resistant to mould infestation, crop rotation and application of fungicides. Besides conventional techniques, innovative approaches are now increasingly applied, such as the outputs of genetic engineering or the use of so-called biocompetitive factors. For example, cereals deliberately infected with spores of competitive non-toxinogenic strains of *Aspergillus flavus* contain only traces of aflatoxins.

Postharvest measures

The successful protection of crops against fungal attack begins at the moment of harvest. Crops must be harvested at full maturity, when their humidity is low, or must be dried before storage. With regard to the manufacturing industry, all types of physical methods can extend food shelf life, such as pasteurisation, sterilisation, cooling, vacuum packaging or irradiation, and can be seen as a precautionary measures. In practice chemical methods are also occasionally used, and include the use of various preservatives, such as sorbic, benzoic or propionic acids.

Detoxifying

Although the above discussed preventive measures can help to reduce the occurrence of mycotoxins in the human food chain, exclusion of toxinogenic microflora is technically impossible and in many cases it is necessary to detoxify and decontaminate large amounts of crop or feed in order to protect the health of consumers or livestock, and to prevent economic losses. In practice, detoxification or decontamination procedures include degradation of mycotoxins to inactive products, prevention of new toxic products formation and maintaining the nutritional value of the material. Generally, there are three possible solutions based on physical, chemical or biological principles.

Physical methods A specific feature of contamination of agricultural crops with mycotoxin is the extreme homogeneity of their distribution. Concentrations of mycotoxins in infected grains or seeds may reach as high as tens of mg/kg. For this reason, an effective measure to reduce the risk of food contamination is mechanical removal of attacked particles. A decrease in the content of mycotoxins may be also achieved by washing or milling the infected grains.

Heat treatment of contaminated materials decreases mycotoxin concentrations in many cases. Aflatoxins are especially thermostable and a significant decrease in their content occurs at temperatures of about 200 °C. Ergot alkaloids and zearalenone are also very stable at high temperatures.

The effective removal of aflatoxins from oilseed meals can be achieved by using different solvents (such as acetone or ethanol), but this process is cost-consuming and also leads to a loss of nutrients and may affect the organoleptic properties of oilseed meals. Aflatoxin residues which may be present in crude vegetable oils are removed during refining.

The use of different sorbents for the removal of mycotoxins has been the subject of intense studies. Aflatoxins may be removed from milk, cream or peanut oil using hydrated calcium aluminosilicate clay or bentonite. These mineral materials are sometimes added directly to feed to immobilise mycotoxins in the digestive tract of livestock (reducing the transfer into the blood stream). For example, the addition of hydrated calcium aluminosilicate to the feed of dairy cows decreases excretion of aflatoxin M_1 to milk by 24–44%. Experiments with the immobilisation of zearalenone have been promising. However, the question remains as to whether the use of these chemisorbents does not reduce the intake of some essential minerals (such as copper, zinc and iron) and water-soluble vitamins.

Chemical methods The greatest attention has been paid to the possibility of chemical degradation of aflatoxins. The most important method is decontamination with ammonia. Other agents tested were sodium hypochlorite and hydrogen peroxide. Practical application of the last two agents is problematic due to unwanted oxidation of a number of treated material components. Aflatoxins B_1 and G_1 and patulin are partially degraded by sulfur dioxide.

Biological methods Biological detoxification in practice means biotransformation or biodegradation of mycotoxins by the action of enzymes, which generates metabolites that are non-toxic or less toxic than the starting toxin and can be easily eliminated from the body. Bacteria studied in this respect are *Flavobacterium aurantiacum* and some fungi of the genus *Rhizopus*. Biotechnological methods have many advantages over chemical methods, as they do not use aggressive agents deteriorating material properties and are often beneficial as they improve the digestibility and usability of proteins. Promising results in detoxification of trichothecenes, for example, have been brought about by biotechnological methods utilising microflora of the gastrointestinal tract of monogastric animals.

12.3.2 Bacteriotoxins

In terms of chemical food contamination, mycotoxins undoubtedly represent the most monitored group of pollutants, but many bacterial pathogens that produce diseases similarly have the ability

to produce toxins (toxigenesis). Two main groups of bacteriotoxins are recognised, lipopolysaccharides (lipooligosaccharides) and proteins (polypeptides). Lipopolysaccharides (lipooligosaccharides) are **endotoxins** associated with the cell walls of Gram-negative bacteria. Proteins (polypeptides) are **exotoxins**, which are usually secreted by bacteria or released by lysis of bacterial cells and act at a site removed from bacterial growth. Exotoxins can be further classified by toxic effects at the site of damage. The names of toxins indicate the location for their activity:

- **enterotoxins** are toxins that attack the intestinal mucosa and cause diarrhea are generated in the intestines
- **cytotoxins** are toxic to cells of exposed organisms
- **neurotoxins** interfere with the transmission of nerve impulses.

Bacterial protein toxins are the most powerful human poisons and their production is generally species specific. Usually, virulent strains of the bacteria produce toxins, while non-virulent strains do not. Most cases of food poisoning are infections caused by bacteria, such as *Salmonella* and *Campylobacter*; less common, but fatal, are intoxications caused by bacteria *Clostridium botulinum*. Fortunately, bacterial toxins occur relatively rarely in the human diet and generally accepted hygienic limits have not been established.

The most effective way to minimise food-borne poisoning caused by bacterial toxins is to respect the principles of HACCP (Hazard Analysis Critical Control Points) in food production and handling. Uniform principles for food producers and their responsibility for food safety are governed by Regulation (EC) No. 852/2004 concerning hygiene in production, distribution and sale of food and introduction of critical control points (HACCP) as preventive tools for ensuring food safety. For foods of animal origin, these requirements are further specified in Regulation (EC) No. 853/2004.

12.3.2.1 Botulotoxins

Botulinum toxins (botulotoxins) produced by bacteria *Clostridium botulinum* are extremely toxic proteins consisting of a sequence of about 1300 amino acids, but not all strains of *C. botulinum* produce these toxic metabolites. Seven toxigenogic types of bacteria exist, each producing a distinct form of botulinum toxin that may cause botulism. The toxins are designated A–G, most of which have several subtypes. Particularly toxic are types A, B, E and F. In Europe, the most frequently occurring toxin is the type B toxin. In the United States, the type A toxin is the most significant cause of botulism. Botulinum toxins may enter the human body by ingestion of toxin from foods (food-borne botulism), but also by certain other pathways.

Production of botulinum toxins proceeds under anaerobic conditions, and the optimum conditions for their formation in foods are pH 4.8–8.5 and temperature of about 30 °C, but toxins are also produced at lower temperatures. Botulinum toxins have been known since the 18th century when they were described as a 'sausage poison', because they arose from improperly handled or prepared meat products. Even nowadays, they can be found in very rare cases in non-acidic canned products, such as sausages and some other meat products.

Owing to the extreme danger of botulinum toxins (intoxication might be lethal), the possibility of preventing their occurrence in foods has been studied extensively. The most important aspect of botulism prevention is proper food handling and preparation. The spores of *C. botulinum* can survive boiling (100 °C at normal pressure) for more than 1 h although they are killed by autoclaving. Because the toxin is heat-labile boiling or intense heating of contaminated food (e.g. heating at 80 °C for 10 min or cooking at 100 °C for several seconds) will inactivate the toxin. Food containers that bulge may contain gas produced by *C. botulinum* and should not be opened or tasted. Nitrites have inhibitory effects (see Section 11.2.1.3.2).

12.3.2.2 Other bacterial toxins

Toxic products may, under certain circumstances, produce a range of bacteria. Their toxins might be present in food at the time of consumption, as in the case of some metabolites of the genus *Staphylococcus*, especially *S. aureus*, which produces seven neurotoxins (toxic proteins). The most common cause of staphylococcal food poisoning are toxins of types A and D (individually or in combination). *Clostridium perfringens* (of type A–E) produces four major toxins (α, β, ε and ι). The cause of food poisoning (gastroenteritis) is mainly *C. perfringens* of the type A producing toxin α, which shows the activity of phospholipase C. Another cause of food poisoning (gastroenteritis) is often bacteria *Bacillus cereus*.

In non-invasive infections, bacteria reproduce and produce toxins in the digestive tract. Most attention in this regard is given to toxins produced by the bacteria *Vibrio cholerae* (the causative agent of cholera), the strain 0157:H7 of *Escherichia coli* (a cause of poisoning called colitis) and, finally, to substances produced by bacteria of the genus *Salmonella*, especially *S. enteritidis* PT4 (*Salmonella enterica* ssp. *enterica* serovar *enteritidis*), which causes diarrhea (the poisoning is called salmonellosis, which in people at risk, such as infants, small children and the elderly, can become very serious). Often a cause of diarrhea known as schigellose are toxins of bacteria *Shigella sonnei*. Previously rare, but more often encountered today is campylobacteriose, caused by bacteria *Campylobacter jejuni* and other infections, such as jersiniose (*Yersinia enterocolitica*), vibriose (*Vibrio parahaemolyticus*) and listeriose (*Listeria monocytogenes*). The pathogens present in the food may also include some viruses, such as the hepatitis A virus that causes infectious hepatitis in humans.

12.4 Persistent organohalogen contaminants

An undesirable consequence of some anthropogenic activities in the 20th century was, and still is, a series of environmental contaminations caused by persistent organic pollutants (POPs),

such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzodioxins (PCDD). Persistence in the environment is a characteristic property of certain chlorinated aromatic and cyclodiene pesticides. These compounds are characterised by high resistance to oxidation and hydrolysis (particularly the chlorine bound to the aromatic ring carbon is very stable); therefore their degradation and biotransformation are very slow. Generally, resistance to photolysis and biodegradation increases with the number of halogen atoms in the molecule. POPs are non-ionisable substances with only very limited solubility in water. Their ability to penetrate through the phospholipid structures of biological membranes (which limits their bioavailability) leads to bioaccumulation in tissue lipids of living organisms, including humans. An exception is perfluorinated contaminants that bind to proteins (see Section 12.4.3.2). The bioaccumulation process involves the biological sequestering of a substance that enters the body either by respiration, food intake or dermally. The result of these processes is the growth of its concentration in the sequestering medium (lipids) compared with the surrounding environment. The degree of bioaccumulation depends on the speed and type of intake, the rate of elimination from the body, the extent of biotransformation, lipid content in the organism and environmental factors. Bioaccumulation in food chains is associated with the risk of chronic toxic effects (even if concentrations of POPs in the environment are not high), which increases with the value of the residence time of the pollutant in the body (non-toxic chlorofluoro substituted aliphatic hydrocarbons, although persistent and causing undesirable phenomena in the atmosphere are not classified as POPs). After their release into the environment, POPs can quickly be transported great distances through a series of evaporation, transportation and condensation cycles. As a result of global circulation patterns and the low evaporation rates in cold climates, POPs tend to accumulate in arctic regions where they bioaccumulate in living organisms. A prerequisite is that the vapour pressure of the pollutant is lower than 1000 Pa. The burden of the Arctic ecosystem and mountain areas is particularly worrying. For example, in Canada's Arctic region, the level of POPs in the breast milk of Inuit women has been found to be up to nine times higher than in women living in southern Canada.

Because of their ability to be transported long distances globally by water and air, controlling POPs require global commitment and action. Accumulaton of information on the risks arising from the presence of POPs in the environment (usually as the result of exposure, interference with hormonal processes and damage to some organ systems, neurotoxicity, immunotoxicity, mutagenicity and carcinogenicity) was initiated in 1995 (by the United Nations Environment Programme, UNEP) in a series of intergovernmental negotiations, which culminated in 2001 in the adoption of the Stockholm Convention, which obliges signatory countries to restrict the selected substances, their production, use and release into the environment. The Stockholm Convention requires, in the case of intentionally produced substances (some classic organochlorine pesticides and the production of their intermediates) their prohibition or phasing out of the production, use, export or import (except DDT, which can still be used in the fight against malaria, until safer effective means are found). The case of untargeted

Table 12.25 Properties of substances with a high potential for bioaccumulation (indicative figures).

Criteria[a]		Criteria values
Bioaccumulation	log K_{OW}	>5
	or bioaccumulation factor	>5000
Persistence (half-life)	Water	2 months
	Soil	6 months
	Sediment	6 months
Transport to distant localities (half-life)	Air	>2 days

[a] According to the Stockholm convention.

produced POPs (primarily polychlorinated dioxins and furans) highlights the need to reduce and gradually eliminate the emissions into the environment.

Table 12.25 briefly summarises the basic criteria that must be met by contaminants to be classified as POPs. Table 12.26 lists the original POPs included in the Convention, along with other high-risk pollutants, which were included in 2009 in the light of the (then) latest information. Also linked with efforts to restrict the movement of persistent substances in the environment is the implementation of the new EU policy REACH (Registration, Evaluation and Authorisation of CHemicals), which represents a new chemical control system to ensure that, by 2020 at the latest, only compounds with known properties are used in a manner that protects the environment and human health.

The following sections present, from the perspective of human dietary exposure, the major groups of POPs, representing industrially manufactured products and intermediates and untargeted pollutants. Classical persistent organochlorine pesticides are included, together with modern pesticides, in Section 12.6

12.4.1 Polychlorinated biphenyls

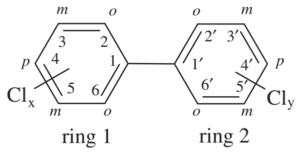
Polychlorinated biphenyls (PCBs) belong to a broad family of man-made organic chemicals known as chlorinated hydrocarbons. They are probably one of the world's most watched groups of POPs. PCBs were manufactured from 1929 until 1979, when their manufacture was banned. They show a range of toxicities and vary in consistency from thin, light-coloured liquids to yellow or black waxy solids. Owing to their low acute toxicity, non-flammability, chemical stability, high boiling points and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications, including electrical, heat transfer and hydraulic equipment, were used as lubricants, additives to pesticides, plasticisers in paints, plastics and rubber products, in pigments, dyes and carbonless copy paper and in many other industrial applications. Table 12.27 summarises the basic facts of

Table 12.26 Compounds in the Stockholm Convention list of POPs.

Persistent organic polutant	Pesticides	Industrial products and intermediates	Non-targeted products of anthropogenic activity
Aldrin	+		
DDT	+		
Dieldrin	+		
Endrin	+		
Heptachlor	+		
Chlornan	+		
Mirex	+		
Toxaphene	+		
Hexachlorobenzene (HCB)	+	+	+
Polychlorinated biphenyls (PCBs)		+	+
Polychlorinated dibenzodioxins (PCDD)			+
Polychlorinated dibenzofurans (PCDF)			+
Hexabromobiphenyl (HBB)[a]		+	
Tetrabromo biphenyl ether (tetraBDE) and pentabromo biphenyl ether (pentaBDE)[a]		+	
Hexabromo biphenyl ether (hexaBDE) and heptabromo biphenyl ether (heptaBDE)[a]		+	
Pentachlorobenzene[a]	+	+	+
Lindane (γ-hexachlorocyclohexane, γ-HCH)[a]	+		
α-Hexachlorocyclohexane (α-HCH)[a]	+		+
β-Hexachlorocyclohexane (β-HCH)[a]	+		+
Chlordekone[a]	+		
Perfluorooctanesulfonic acid (PFOS) and its salts[a]		+	+

[a] POPs in the list of the Stockholm convention in 2009.

the history of the PCBs production and use. Among other things, there is a documented sequence of new information about the properties of PCBs and continuity of measures that resulted in the restriction of their use and ban.



12-130, polychlorinated biphenyls

12.4.1.1 Structure and nomenclature

PCBs are substances of molecular formula $C_{12}H_{10-(x+y)}Cl_{x+y}$ (**12-130**), where $x+y = 1$–10, $x =$ number of Cl atoms in ring 1, $y =$ number of Cl atoms in ring 2.

Theoretically, 209 individual compounds, called **congeners** (members of the same group), each of which has its own systematic name, can be derived from the basic biphenyl skeleton. Specification of the structure of the individual PCBs (number of chlorine atoms and their position in the aromatic rings of biphenyl) is shown in Figure 12.46. To simplify communication at the level of professional and legislative practice, a uniform nomenclature is used, proposed at the international level (by the International Union of Pure and Applied Chemistry, IUPAC), where congeners have assigned numbers of from 1 to 209 in order of increasing numerical index (2, 3, 4, 22′, 23, 23′, etc.). Existing three isomeric monochlorobiphenyls do not belong to the polychlorinated group of compounds, but are commonly classified as PCBs for practical reasons.

Table 12.27 History of PCB on a global scale.

Year	Events
1881	First synthesis of PCBs
1929	Start of industrial production of PCBs (Monsanto Chemical Company, USA)
1936	Negative health effects in persons who come into contact with the PCBs were demonstarted
1966	Evidence of PCBs occurrence in tissues of animals and humans (Sweden), PCBs classified as global environmental contaminants
1968	Mass poisoning of more than 1000 people by contaminated rice oil (Yushō disease, Japan)
1971	Voluntary restrictions on the distribution of products based on PCBs by Monsanto (only supplied for controlled applications)
1973	WHO working group charged with complex evaluating of PCBs; proposal of restrictions on use of PCBs; restrictions on the manufacture, sale and use of PCBs in OECD countries
1976	Prohibition of the manufacture, processing, distribution and use of PCBs by the US Congress (with the exception of closed systems)
1978	Restrictions on the use of PCBs (except for closed systems) in most advanced industrial countries
1979	Mass poisoning of more than 2000 people by contaminated rice oil (Yu-cheng disease, Taiwan); federal law banned US production of PCBs
1983	Voluntary cessation of production PCBs by the German company Bayer
1987	Ban on all new applications of PCBs in 24 OECD countries; the intensification of solving problems connected with the replacement of the existing PCBs and providing suitable means of PCBs disposal

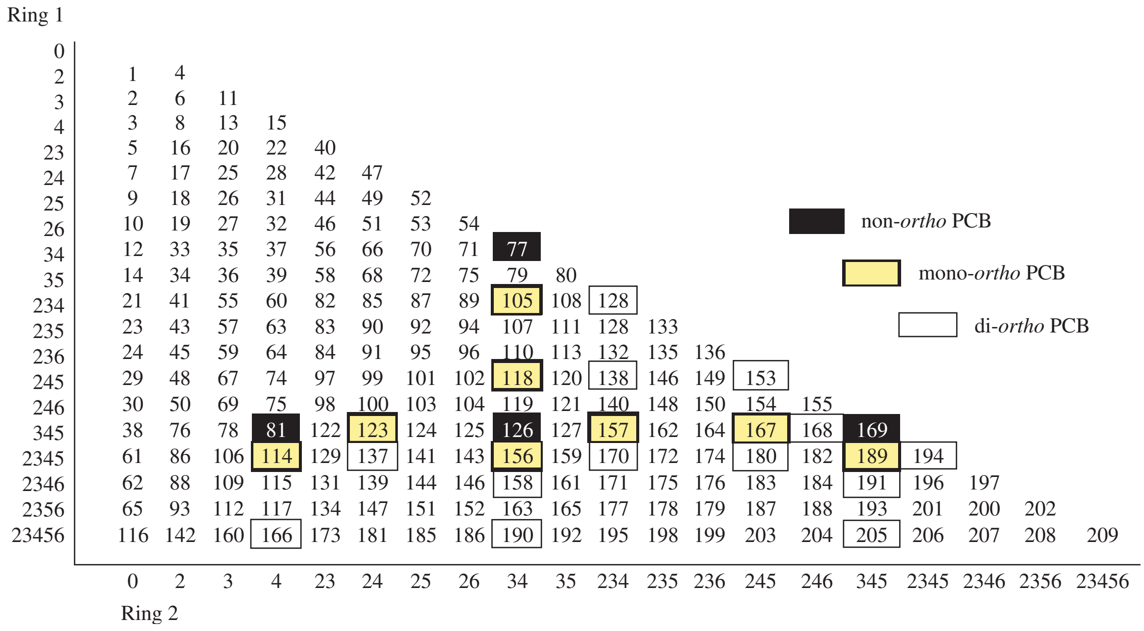


Figure 12.46 Structure of PCB congeners and their labelling according to IUPAC (the axes show the positions of chlorine atoms in the biphenyl aromatic rings, white rectangles = non-*ortho* PCB, grey rectangle = mono-*ortho* PCB).

Table 12.28 Characterisation of groups of PCBs.

Molecular formula of isomer groups	Trivial name	Basic relative molecular weight[a]	Average relative molecular weight[b]	Chlorine content (% w/w)	Number of isomers
$C_{12}H_9Cl$	MonoCB[c]	188.0	188.7	19	3
$C_{12}H_8Cl_2$	DiCB	222.0	223.1	32	12
$C_{12}H_7Cl_3$	TriCB	256.0	257.6	41	24
$C_{12}H_6Cl_4$	TetraCB	289.9	292.0	49	42
$C_{12}H_5Cl_5$	PentaCB	323.9	326.4	54	46
$C_{12}H_4Cl_6$	HexaCB	357.8	360.9	59	42
$C_{12}H_3Cl_7$	HeptaCB	391.8	395.3	63	24
$C_{12}H_2Cl_8$	OctaCB	425.8	429.8	66	12
$C_{12}HCl_9$	NonaCB	459.7	464.2	69	3
$C_{12}Cl_{10}$	DecaCB	493.7	498.7	71	1

[a] Isotope ^{35}Cl (relative atom weight = 34.969), ^{12}C (12.011) and ^{1}H (1.008).
[b] Corresponds to the natural occurrence of C, Cl and H isotopes.
[c] CB=chlorobiphenyl.

Planar congeners containing up to two substituents in the *ortho* position are indicated, which are further discussed in the context of their biological activity relative to dioxins. Also highlighted are so-called **indicator congeners** that are employed as markers of contamination. Table 12.28 presents the characterisation of the individual PCBs resulting from the number of bound chlorine atoms.

In unsubstituted biphenyl phenyl, the residues can freely rotate around the single bond connecting the aromatic rings (the planar conformation with the lowest energy content is preferred), however, the conformation of PCBs depends on the number and position of substituting chlorine atoms (they are bulkier than hydrogen atoms in the parent compound), which may prevent rotation. Substituents in the *ortho* position play a particularly important role. At a high degree of substitution, the relative position of aromatic rings is orthogonal and conformations of molecules determine not only the physico-chemical properties of individual congeners, but also their toxic effects.

12.4.1.2 Production

PCBs were manufactured by controlled chlorination of biphenyl catalysed by ferric chloride or iron filings. Technical mixtures used in practice (a chlorine content of 21–68% w/w) were obtained by fractional distillation of the neutralised reaction mixture. The presence of individual congeners in the reaction mixture depends on the conditions of chlorination. Chlorine (substituent of the first order) preferentially enters the *ortho* and *para* positions of biphenyl. Therefore, the presence of certain congeners in technical mixtures of PCBs is very unlikely and they only occur as minor components. In most products only about 100–140 congeners are found in significant concentrations (>0.05% w/w), but technical mixtures with the same chlorine content from various manufacturers are never completely identical. The largest share of manufactured PCBs in the world falls to technical mixtures with the trade name Aroclor, manufactured by Monsanto (USA). A representation of different groups of congeners in these products is shown in Table 12.29 (where the chlorine content in % w/w specify the last two digits in the Aroclor designation). The trademark Aroclor included not only technical PCB mixtures, but also a wide range of products based on polychlorinated compounds, such as Aroclor 5460, which was a mixture of polychlorinated terpenes. Other products also existed on the market, with trade names, for example, Clophen, Phenochlor, Kanechlor, Pyralene and Fenclor. It is estimated that, before their manufacture was halted, global production in countries producing technical PCB mixtures (including the United States, Canada, United Kingdom, Germany, France, Japan, Italy, Sweden, Norway, Finland, Switzerland, Spain, the USSR, Czechoslovakia and Poland) was 1.2×10^6 tons, with most of this amount (about 93%) produced in the United States.

12.4.1.3 Properties

12.4.1.3.1 Physical and chemical properties

The so-called advantageous physico-chemical properties (Table 12.29) which led to widespread use of PCBs are summarised in the following list:

- thermostability
- photostability (resistance to sunlight)
- non-combustibility
- chemical inertness (resistance to acids, bases, oxidation and reduction)

Table 12.29 Distribution of homologues in technical PCB mixtures Aroclor (% w/w) and their lipophilicity.

Number of chlorine atoms	Aroclor 1221	1232	1242	1248	1254	1260
0	10	-	-	-	-	-
1	50	26	1	-	-	-
2	35	29	13	1	-	-
3	4	24	45	22	1	-
4	1	15	31	49	15	-
5	-	-	10	27	53	12
6	-	-	-	2	26	42
7	-	-	-	-	4	38
8	-	-	-	-	-	7
9	-	-	-	-	-	1
Solubility in water[a]	15 000	1400	240	52	12	3
K_{ow}	0.65	1.7	3.5	6.4	12	22

[a]In μg/l at 25 °C.

- low volatility
- high permittivity (dielectric material, electrical insulators)
- excellent heat transfer properties
- excellent miscibility with organic solvents
- broad interval of melting points (optional consistency)
- relatively high boiling points.

12.4.1.3.2 Persistence in living organisms

Bioaccumulation and bioconcentration are the major events that contribute to the overall occurrence of POPs in biota. As a result, the content of POPs in biota is higher than in the surrounding components of the environment, for example water. The extent of these processes depends on the K_{OW} values. PCBs, as well as other halogenated POPs, are significantly lipophilic substances that have high affinity for the fat in organisms. Table 12.30 represents the average log K_{OW} values and bioconcentration factors (BCF) for fish, which are related by the equation: $BCF = 0.048K_{OW}$. Along with bioconcentration, processes leading to decrease of PCB levels in the organism may also occur (such as metabolic transformation and spawning), so the real level of congeners estimated from bioconcentration factors may sometimes differ significantly. It should be noted that the classification of organic contaminants as POPs considers the bioconcentration factor higher than 5000, which some low-chlorinated PCBs do not meet.

12.4.1.4 Occurrence and main sources

PCBs are found globally in virtually all parts of the environment. Owing to the restrictive measures which have been adopted, their

Table 12.30 Bioconcentration of PCBs in fish.

Trivial names of congener groups	log K_{ow}	Bioconcentration factor (BCF)	Trivial names of congener groups	log K_{ow}	Bioconcentration factor (BCF)
MonoCB	4.7	2500	HexaCB	6.7	250 000
DiCB	5.1	6300	HeptaCB	7.1	630 000
TriCB	5.5	16 000	OctaCB	7.5	1600 000
TetraCB	5.9	40 000	NonaCB	7.9	4000 000
PentaCB	6.3	100 000	DecaCB	8.3	1000 000

concentrations are declining gradually, but some particularly persistent congeners are doing so very slowly. Generally, the composition of PCB mixtures is often significantly different from the profile of PCBs in the environment, especially in biotic samples, where, in addition to differences in physico-chemical properties of individual congeners, often the very different scope of their biodegradation and bioaccumulation is also reflected. To harmonise the communication and interpretation of contamination, seven indicator congeners were selected, these being: PCB 28 (2,4,4′-tetrachloro-), 52 (2,2′,5,5′-tetrachloro-), 101 (2,2′,4,5,5′-pentachloro-), 118 (2,3′,4,4′,5-pentachloro-), 138 (2,2′,3,4,4′,5′-hexachloro-), 153 (2,2′,4,4′,5,5′-hexachloro-) and 180 (2,2′,3,4,4′,5,5′-heptachlorobiphenyl). Sometimes only six PCB markers are followed without congener 118. These congeners are present in technical mixtures in relatively high concentrations and are characterised by medium to high persistence. Sometimes a systematic monitoring includes PCBs 8, 18, 31, 44, 66, 70, 74, 99, 128, 149, 163, 170, 183, 187 and 194 (Figure 12.46). The impetus for the introduction of indicator congeners was a result of the so-called Belgian crisis in 1999, when a feed that was highly contaminated by Aroclors 1254 and 1260 was distributed to many poultry farms and indicator congeners were used for the rapid retrieval of contaminated samples.

The evaluation of health risks is based on the WHO proposal from 1997, which divided PCBs into two groups based on their structure and related toxicity. The first group consists of PCBs able to bind to the Aryl Hydrocarbon Receptor (AHR),[7] which cause toxic effects similar to the toxic effects of dioxins. Among these, the so-called dioxin like PCBs (DL-PCBs), include a total of 12 congeners, four non-*ortho* and eight mono-*ortho* PCBs; the most important in terms of the toxic potential is congener 126 (3,3′,4,4′,5-pentachlorobiphenyl). The second group consists of the non-dioxin like PCBs (NDL-PCBs), which are characterised by different (not necessarily identical) toxicity profiles. The above mentioned seven indicator congeners represent a group of mostly NDL-PCBs, except for mono-*ortho* congener 118.

[7] An Aryl Hydrocarbon Receptor (AHR) is a cytosolic transcription factor in an inactive form bound to co-chaperones. After binding to compounds such as polychlorinated biphenyls and polychlorinated dibenzo-*p*-dioxins, chaperones (special proteins that assist the non-covalent folding/unfolding of many proteins in their correct spatial arrangement) dissociate and AHR is translocated into the cell nucleus, which leads to changes in gene transcription.

12.4.1.4.1 Release into the environment

Owing to their low acute toxicity, until the beginning of the 1970s PCBs were seen as biologically inactive substances. In principle, four types of situations can be identified that lead to PCBs being released into the environment:

- use in open systems (additives for paints and varnishes, plastics, pesticide formulations, inks and print colours);
- storage at authorised landfills (poor technical background);
- illegal or technically imperfect disposal (direct release into the environment and inefficient combustion);
- accidental releases during manufacture, transportation or operation of hydraulic and heat transfer equipment, transformers and capacitors.

It is estimated that about 4×10^5 tons of technical mixtures are released into the external environment, which corresponds to about 31% of worldwide production. Approximately 65% of the total stock of PCBs is now rigorously recorded and used in so-called closed systems. The remaining 4% of PCB production have already been disposed of by safe incineration. Table 12.31 provides an estimate of the distribution of PCBs that leaked into the environment in the United States up until 1981, in the period when the risks associated with these substances were known and legislative measures existed. European studies also state similar values of relative ratios. It is expected that the largest quantity of PCBs is deposited in the North Atlantic. On the mainland, the most important reservoirs of PCBs are freshwater sediments, which are also the primary source of contamination of the food chain of fish and humans. In general, higher concentrations of PCB residues are found in the vicinity of urban and industrial sites and so-called point sources (landfills of manufacturing companies, places of uncontrolled releases or accidents) than in sparsely populated areas and oceans.

Table 12.31 Typical environmental distribution of PCBs at the beginning of the 1980s.

Components of environment	Weight of PCBs (tons)	Components of environment	Weight of PCBs (tons)
Atmosphere	18	Vegetation	1000
Water (rivers and lakes)	20	Wild animals	0.3
Freshwater sediments	4000	Livestock	0.6
Freshwater animals	15	People	5
Soil – natural	1000	Oceans – water and animals	6000
– sewage sludge	4800	– sediments	1000

12.4.1.4.2 Transport between parts of the environment

Transport between the non-living (abiotic) and living (biotic) parts of the environment is a dynamic process that is influenced by many factors. In Table 12.32 the typical concentration of PCBs that are found in various abiotic ecosystems are illustrated. Table 12.33 gives similar orientation data for biotic parts of the environment.

Atmospheric transport

For PCBs and many other environmental contaminants, the most important transport medium is the atmosphere. Especially mobile are the low-chlorinated (more volatile) congeners. After the transition from an aquatic or terrestrial ecosystem, PCBs can be transmitted through the air in gaseous form or sorbed onto dust particles. The length of stay of individual congeners in this form is in an approximate range of 40–70 days, which is influenced, *inter alia*, by the number of hydroxyl radicals ($HO^{\bullet}$). Thanks to long-distance transmission in the atmosphere and re-deposition (wet or dry), PCBs are found in soils, sediments and on the surface of vegetation, even in outlying places far from the primary emission source. The average atmospheric concentrations of PCBs in urban areas are usually much higher than in rural areas or over oceans, and the highest concentrations can be found in the Arctic regions. As a result of the increase in vapour pressure at elevated temperatures, PCB concentrations in the atmosphere are up to five times higher in the summer months.

Contamination of aquatic ecosystem

Ocean waters contain probably the most significant portion of the total quantity of PCBs emitted to the environment (Table 12.32). The content of PCBs (and other organohalogenated substances) in the hydrosphere is in equilibrium with the atmosphere and bottom sediments.[8] Owing to their low solubility and high affinity for the organic components, high-chlorinated PCBs ($>Cl_5$) are primarily accumulated. The PCBs contents of sediments range from

[8]The equilibrium between water and sediments can be described by the equation: $C_S = K_P.C_W$ where C_S = concentration of PCBs in sediments, C_W = concentration of PCBs in water and K_P = equilibrium constant that reflects the character of sediments. To calculate the K_P value, tabulated values of K_{OC} (soil adsorption coefficient) can be used and K_P values calculated using the equation: $K_P = K_{OC}.TOC$ (total organic carbon). For a description of the behaviour (or distribution) of individual PCB congeners between water and sediments a linear relationship between K_{OC} and tabulated K_{OW} values can be used: $\log K_{OC} = 0.85 \log K_{OW} + 0.13$. The relationship is valid within the range of values of $\log K_{OW} = 4.5–7.7$.

Table 12.32 Typical concentrations of PCBs in the abiotic environment in the beginning of the 1980s.

Part of environment	Location and environment part specification	Concentration	Units
Air	Urban agglomerations	0.5–30	ng/m^3
	Rural areas	0.1–5	
	Over the Atlantic Ocean	0.05	
	Around the factory transformers	20–6000	
Water	Rivers – background values	<0.5	ng/l
	– mean contamination	<50	
	– strong contamination	<500	
	Atlantic Ocean	0.3–8	
	Mediterranean Sea – coast	13	
	Rain – countryside	1–50	
	– urban agglomerations	10–250	
Soil	Background values	1–5	μg/kg
	Around the factory transformers	15–18 000	
Sediments	Lakes – background values	0.1–5	μg/kg
	Rivers – normal load	8–20	
	– in the vicinity of PCB leaks	up to 60 000	

Table 12.33 Typical concentrations of PCBs in the biotic environment in the beginning of the 1980s.

Organism	Concentration (mg/kg fat)	Organism	Concentration (mg/kg fat)
Vegetation	0.001–0.01	Marine mammals	0.1–1000
Aquatic zooplankton	0.005–2.0	Birds	0.1–1000
Aquatic invertebrates	0.005–10	Eggs of birds	0.05–500
Fish	0.01–25	People	0.1–50

0.001 mg/kg for normal loads up to 1 mg/kg in heavily polluted areas. PCBs can, however, be found in virtually all other abiotic and biotic components of the aquatic ecosystem, such as plankton, arthropods, molluscs, fish, seabirds and mammals (Table 12.33). There is a significant bioaccumulation of PCBs in the introductory component of a food chain – in this case plankton, where concentrations of PCBs can be higher than in water by several orders of magnitude. Extensive leakages of PCBs into the environment are usually associated with ecological accidents, accompanied by contamination of groundwater. Under these circumstances, the concentrations of PCBs in water can be as high as hundreds of ng/l, which does not correspond to the solubility of technical mixtures of PCBs in water in some cases, but this phenomenon can be ascribed to solubilising effects of various surfactants, such as humic acids.

Contamination of soil and vegetation

The main source of contamination of soil is dry and wet atmospheric deposition. Increased findings are mostly local and related to specific point-source pollution. The extent of PCBs binding to soil particles increases with an increasing proportion of organic carbon, but PCBs may also undergo biodegradation by soil microflora, and there is also the possibility of eventual transfer of PCBs from contaminated soil into plants. This risk may seem especially timely in addressing the disposal of sewage sludge, but significant translocation of PCBs, even in strongly contaminated soil, to the above-ground parts of plants does not occur (with the exception of some low-chlorinated PCBs) and sorbed contaminants remain in the surface layers of the root system. A specific accumulation of PCBs may occur, for example, in carrots, and is associated with a higher concentration of lipophilic carotenoid pigments. Contamination of the aerial parts of vegetation with PCBs is mainly related to atmospheric pollution and, to some extent, to re-emissions of volatile PCBs from soil.

Contamination of animals

The fate of individual congeners of PCBs and their occurrence in various food chain components are different, sometimes very significantly. To animal organisms, PCBs penetrate from outside via food and other sources, but in the aquatic environment penetration from the surrounding medium also plays an important role. Some congeners of PCBs have significant bioconcentration potential. Additionally, biomagnification takes place in predatory organisms. It is reported that biomagnification in fish occurs only when the values of the bioconcentration factor (BCF) exceed the value of 1.15×10^5 (Table 12.30). The PCB burden of the prey is transferred to the predator. Differences in concentrations of PCBs and other POPs between the input and terminal components of a food chain as a result of biomagnification can be up to several orders of magnitude. For example, fish that consume large quantities of zooplankton magnify the PCB concentration in the body. This leads to bioaccumulation factors (BAF) as high as 2.8×10^6 in predatory fish species. Reptiles, birds and mammals, including humans who eat the fish, further accumulate PCBs.

Other processes may proceed simultaneously with bioaccumulation, such as diffusion into the surroundings, biodegradation and excretion (e.g. through fish roe or milk in female mammals). In general, the proportion of these events is species-dependent and also depends on the age and sex of an organism and its living conditions. For example, food availability determines the content and composition of body lipids, which represent a significant reservoir of PCBs. Increasing the total weight as well as the amount of fat in the body is reflected as a decrease in levels of contaminants, but in essence this phenomenon is a mere dilution at a constant load.

The spectrum of PCB congeners observed in invertebrate organisms and fish is not too different from the composition of PCB congeners in the surrounding abiotic environment (due to the low metabolic activity). In warm-blooded animals, especially higher vertebrates, the spectrum of PCB congeners can be substantially different as the biodegradable congeners are partially or completely eliminated, while in PCBs substituted in positions 4,4′ or 3,4′,5 preferential bioaccumulation and an increased tendency to bioaccumulate toxic DL-PCBs can be observed. Figure 12.47 illustrates the differences in the PCB profiles between organisms of different trophic levels. In humans, who are at the top of the food pyramid, highly persistent PCB congeners 138, 153 and 180 of the group of NDL-PCB dominate.

Exposure of livestock to PCB leads to accumulation of PCBs in meat, especially in liver and adipose tissue. A significant proportion of accumulated PCBs are transferred to eggs and milk and to the offspring by the mother's milk. The largest transmission range, roughly 50–60%, can be observed in highly persistent PCB congeners 138 and 153. Unwinding the exposure leads to a relatively rapid decline in PCB concentration, typically to 50% of the original amount, but the next elimination stage is actually very slow.

12.4.1.4.3 Biodegradation

Microbial degradation

Biodegradation is a significant way of eliminating low-chlorinated PCBs from the environment. Biotransformation mechanisms and terminal metabolites are different in individual species. Certain strains of bacteria of the genera *Alcaligenes*, *Pseudomonas*, *Nocardia* and others have the ability to metabolise PCBs, as do some eukaryotic microorganisms (such as fungi). The degradation rate decreases with the number of chlorine atoms and, starting from pentachlorobiphenyls, is, relatively, very low.

Dihydrodiols are produced in the initial stage of aerobic degradation of PCBs by dioxygenases (and monooxygenases in some bacteria and fungi). Degradation starts preferably in the less substituted aromatic ring. Dihydrodiols are then gradually transformed into chlorobenzoic acids, inorganic chlorides and lower aliphatic acids. The use of bacteria, fungi or green plants with biodegradation potential is the essence of bioremediation techniques (use of biological agents to remove or neutralise contaminants, as in polluted soil or water). Their use may represent one of the most effective ways to protect the food chain.

Metabolism in vertebrates

PCBs as well as many other xenobiotics are substrates of enzymes found in liver cells. Like other biotransformation enzymes, the monooxygenase enzyme system cytochrome P450 is located

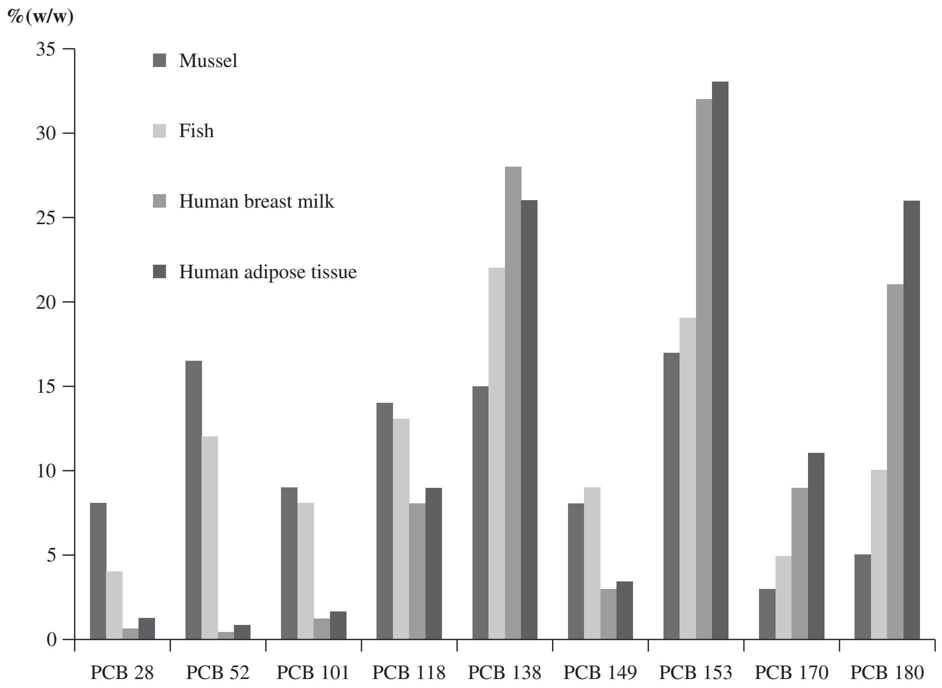


Figure 12.47 Typical relative distributions of selected PCB congeners in organisms at different trophic levels.

primarily in microsomes, artifacts re-formed from pieces of the endoplasmic reticulum in liver cells. Their synthesis increases several fold during long-term (chronic) exposure to a toxic substance.

The first reaction of PCB biotransformation by cytochrome P450 (cytochrome P450, family 2, subfamily B) is the formation of epoxides or hydroxyl derivatives of congeners that form arene oxides with difficulty. The general scheme of metabolic transformations of PCB 101 is illustrated in Figure 12.48.

Congeners with two unsubstituted carbons in the positions *meta* and *para* (such as PCB 52, PCB 95 and PCB 136) can be oxidised at multiple sites. The reactive electrophilic intermediates arene oxides give rise to dihydrodiol-PCBs, polychlorobiphenylols (hydroxy-PCBs) and conjugates with glutathione or adducts with macromolecules (DNA and proteins) and lipids. Dihydrodiols can become the precursors of metabolites with a catechol structure, and may be in equilibrium with hydroquinones and quinones. The major metabolites of PCBs, however, are hydroxylated products. Hydroxy-PCBs may also arise by 1,2-rearrangement of chlorine atoms. Most of these compounds are excreted from the body in a free form or as conjugates (glucuronides or sulfates). From about 50 possible hydroxy-PCB congeners, only five were demonstrated in higher concentrations in the plasma of exposed individuals, either bound to proteins or to transthyretin (carrier of the thyroid hormone thyroxine and retinol binding protein). The affinity of hydroxy-PCBs to lipids in the organism is considerably lower than the affinity of the parent compounds, which leads to their different distribution. PCB congeners with unsubstituted *meta* and *para* positions and chlorinated neighbouring *ortho* and *meta* positions (at least in one ring) are rapidly metabolised. Congeners with free *meta* and *para* positions may become precursors of methylsulfones as a result of sequences of several processes, which involve conjugation with glutathione, degradation to mercapturic acids followed by methylations and oxidations. Methylsulfonyl-PCBs are neutral metabolites with an affinity for lipids that are found in the blood plasma in relatively high concentrations. The key organs of their accumulation are the lungs and liver, where their concentrations may exceed concentrations of the parent PCBs. Some of them are optically active compounds.

12.4.1.4.4 Human exposure

The main source of human exposure (except for professionally exposed persons) is food, which represents more than 90% of the PCB intake. Exposure to PCBs in air, dust and soil is relatively insignificant. Table 12.34 summarises data on the levels found for of six indicator PCBs, which are considered markers of contamination. It is assumed that their contribution to the overall intake of NDL-PCBs is about 50%.

Foods of plant origin contribute to the dietary intake of PCBs to a lesser extent; the degree of contamination varies at levels of ng/kg. Concentrations of PCBs in foods of animal origin (meat, milk and in particular fish) are generally considerably higher, typically μg/kg and their content increases with lipid content. Differences in the profiles of foods of plant and animal origin reflect the ability of the organism to metabolise these pollutants. In foods of animal origin there is lower amount of low-chlorinated PCBs (28, 52, but also 101) as living organisms gradually biotransform these substances and subsequently eliminate them.

The data in Table 12.34 also show the variability of food contamination and higher levels of PCBs primarily in fish oils. The relatively higher levels of PCBs in fish and fish products are caused not only by differences in fat content, but also by different levels of contamination of aquatic ecosystems in various regions. Table 12.35 provides more detailed information, illustrating the

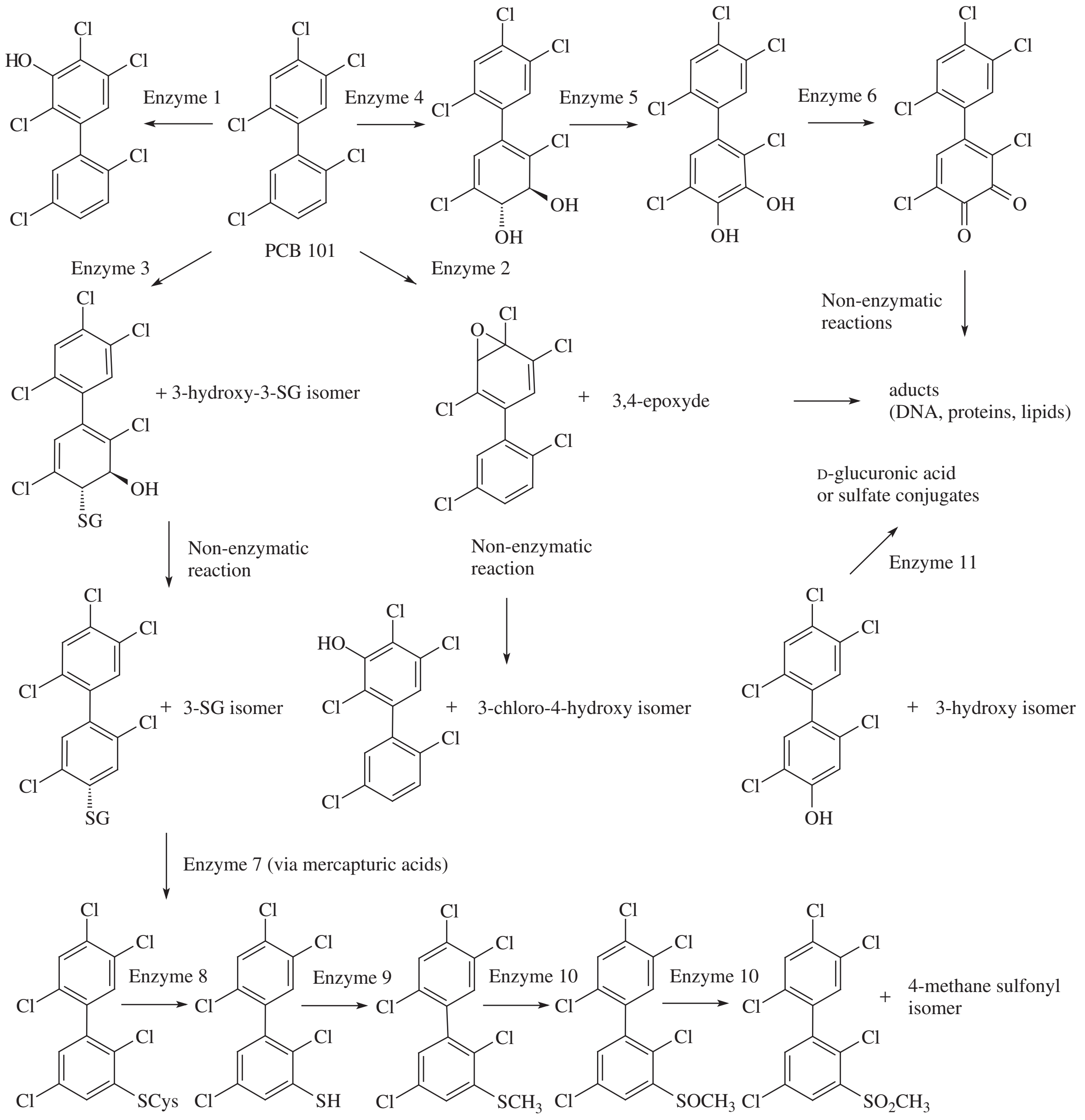


Figure 12.48 Formation of PCB 101 metabolites. Enzyme 1 = CYP2B (cytochrome P450, family 2, subfamily B) in rodents, enzyme 2 = CYP2B, CYP2C and CYP3A (e.g. CYP2B1 in rodents and CYP3A4 in humans), enzyme 3 = glutathione S-transferase (= glutathione residue), enzyme 4 = epoxide hydrolase, enzyme 5 = dihydrodiol dehydrogenase, enzyme 6 = peroxidase or autoxidation, enzyme 7 = glutamyl transferase and cysteinyl glycinase (Cys = cysteine residue), enzyme 8 = C-S lyase, enzyme 9 = *S*-adenosylmethionine-dependent methyltransferase, enzyme 10 = FAD-monoases or cytochrome P450, enzyme 11 = UDP-glucuronosyl transferase or phenol sulfotransferase. *Source*: Hofvander L.: Polychlorinated biphenyls and their metabolites in human blood. Method development. Identification and quantification., Department of Environmental Chemistry, Stockholm University, Doctoral Thesis, 2006. Available at: http://su.diva-portal.org/smash/get/diva2:199492/FULLTEXT01 (accessed 18 October 2013). Fig 2.1.

significant contamination of fish from the Baltic Sea (average concentrations are higher by about 50% when compared with the sum of indicator congeners in fish from other areas) and the associated risk of increased dietary exposure of fish consumers. In Baltic fishermen with a significant proportion of local fish in their diet, the average daily exposure to NDL-PCB may reach as high as 80 ng/kg body weight (based on data from the east coast of Sweden). Also interesting is the significantly higher contamination of salmons compared with herrings. For the European population, the average daily intake of total NDL-PCB of 10–45 ng/kg body weight is assumed. In children under 6 years of age (not including breastfed children), this value is 27–50 ng/kg body weight.

The estimated average exposure to NDL-PCB contained in breast milk is up to two orders of magnitude higher for exclusively breastfed children, and is on average 1600 ng/kg body weight. Levels of population exposure to halogenated POPs are often determined on the basis of their concentrations in breast milk. The WHO study carried out in 2001/2002

Table 12.34 Average amounts of indicator PCB congeners in foods in the European market in 2005.

Foods	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Σ6(PCB)
Cereals and ceral products[a]	0.00835	0.00501	0.00189	0.00217	0.00246	0.00141	0.0213
Fruits and vegetables[a]	0.00632	0.00389	0.00262	0.00742	0.0105	0.0191	0.0495
Eggs[b]	0.59	0.41	0.70	1.81	2.00	1.05	6.60
Vegetable oils[b]	0.65	0.35	0.56	1.42	1.51	0.57	5.05
Animal fats[b]	0.13	0.11	0.13	0.63	1.16	0.46	2.61
Fish oils[b]	0.79	3.44	9.18	23.2	25.5	8.09	70.2
Fish and fish products[c]	0.29	0.63	1.64	3.88	4.41	1.63	12.5
Poultry[b]	0.77	0.96	0.89	5.34	2.31	2.03	12.7
Beef[b]	0.58	0.96	0.55	3.74	2.60	1.10	9.53
Pork[b]	0.45	0.66	0.58	1.54	2.34	1.23	6.80
Milk and diary products[b]	1.48	0.99	1.01	2.57	3.21	1.47	10.7

[a] In μg/kg.
[b] In μg/kg fat.
[c] In μg/kg edible portion.

Table 12.35 Comparison of distribution of indicator PCB congeners in fish and fish products (μg/kg fresh weight).

Product	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Σ6(PCB)
Fish and fish products (including Baltic)	0.29	0.63	1.64	3.88	4.41	1.63	12.5
Fish and fish products (excluding Baltic)	0.24	0.55	1.06	2.69	2.74	0.99	8.27
Baltic fish (including herring and salmon)	0.54	1.06	4.44	9.63	12.5	4.73	32.9
Baltic fish (herring and salmon)	0.22	0.38	1.76	4.29	5.8	2.24	14.7
Baltic fish (herring)	0.66	1.27	5.25	11.4	14.5	5.67	38.8
Baltic fish (salmon)	1.52	3.35	13.8	26.6	35.0	11.7	92.0

examined a total of 102 samples of human milk from 26 countries (including 18 European countries). Data on concentrations of 38 congeners for European countries, including 12 DL-PCB, are summarised in Table 12.36. The contribution of non-*ortho* PCB congeners with the greatest toxic potential to the overall content of PCBs was only 0.04%; the main PCBs were congeners 153, 138 and 180, representing more than 65% on average.

12.4.1.5 Health and toxicological assessment

Toxicological evaluation of PCBs is an extremely difficult problem, as the considered organism is always exposed to mixtures of congeners with different physico-chemical and biological properties. It is practically impossible to distinguish the effects of individual toxic congeners or groups. Food of animal origin in particular may contain NDL-PCBs, DL-PCBs and possibly also polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) simultaneously in different proportions. The acute toxicity of PCBs is low and depends on the degree of chlorination of the technical mixture and the species tested. However, dioxin-like congeners (such as 3,3′,4,4′-tetrachlorobiphenyl) elicit a much higher toxicity. Symptoms of PCB intoxication are manifested by wasting syndrome (progressive weight loss, not related to food consumption), skin disorders, lymphoid involution (immunosuppresion), endocrine and reproductive effects (menstrual irregularities and reduced conception rate, early abortion, excessive menstrual haemorrhage and, in males, testicular atrophy and decreased spermatogenesis); PCB mixtures did not cause mutation or chromosomal damage in a variety of test systems, although adducts with DNA, RNA and proteins could be detected (prior metabolisation is necessary). According to the evaluation of the IARC of PCB mixtures, there is sufficient evidence for carcinogenicity to animals, therefore PCBs are classified as being group 2A agents, and are probably carcinogenic to humans.

Table 12.36 Content of PCBs in breast milk from 18 European countries.

PCB congener		Content (μg/kg fat) Mean	Minimum	Maximum	Proportion in %
Non-*ortho*	37	0.025	0.005	0.576	0.01
	77	0.011	0.003	0.173	<0.01
	81	0.005	0.001	0.071	<0.01
	126	0.049	0.012	0.108	0.02
	169	0.031	0.007	0.080	0.01
Mono-*ortho*	28	4.6	0.9	92.1	0.88
	33	0.11	<0.02	0.8	0.02
	60[a]	0.87	0.14	11.3	0.16
	66	2.3	0.39	33.4	0.48
	74	8.4	1.9	29.8	2.72
	105	3.0	0.51	12.2	0.84
	110	0.28	0.05	1.2	0.08
	114	0.70	<0.14	2.0	0.21
	118	12.9	2.2	35.1	4.52
	156	7.1	0.97	27.6	2.76
	157	1.2	0.18	3.0	0.48
	167	2.5	0.38	9.3	0.88
	189	0.68	0.09	3.4	0.22
Di-*ortho*	18	0.13	0.01	0.90	0.04
	52	0.51	0.09	4.6	0.13
	99	9.2	1.6	27.1	2.48
	101	0.86	0.16	3.0	0.28
	128	0.79	<0.16	4.1	0.25
	138	64.0	9.6	286.0	22.19
	141	0.19	0.06	0.60	0.07
	153	81.7	10.9	378.9	27.11
	170	23.5	2.8	148.3	7.16
	180	58.5	6.1	336.9	18.31
	183	7.6	0.83	41.3	2.40
	187	14.4	1.6	62.9	3.84
	194	4.7	0.34	27.2	1.28
	206	0.44	0.07	1.7	0.12
	209	0.26	<0.04	2.9	0.06
	Σ_6 (PCB)[b]	210.1	29.1	1009.1	64.40
	Σ_7 (PCB)[c]	223	31.3	1028.0	68.20

Table 12.36 (*continued*)

PCB congener	Content (μg/kg fat)			Proportion in %
	Mean	Minimum	Maximum	
Σ_{25} (NDL-PCB)	282.8	40.7	1309.0	90.10
Σ_{12} (DL-PCB)[d]	28.2	4.4	67.4	9.90
Σ_{37}(PCB)	311.1	45.1	1374.4	100.00
Σ(138+153+180) x 1.64	334.9	43.7	1643.0	102.60

[a]PCB 60 in only 27 samples.
[b]Σ_6 (PCB) = sum of six indicator congeners: PCB 28, 52, 101, 138, 153 and 180.
[c]Σ_7 (PCB) = sum of six indicator congeners: 28, 52, 101, 118, 138, 153 and 180.
[d]Σ_{12} (DL-PCB) = sum of 12 DL-PCB congeners: PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189.

12.4.1.5.1 Toxic planar congeners

Individual PCB congeners differ by their biological effects. Toxicological studies have shown that some congeners act by the same mechanisms as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), whose toxicity is mediated by interaction with a protein receptor. Other PCBs act by totally different mechanisms.

The assessment of the toxicological risks resulting from exposure to compounds such as PCDD or PCBs uses the system of **toxic equivalents** (TEQs), which considers the different toxicity of individual compounds. TEFs are so-called **toxic equivalency factors** that assign a ranking to various PCB congeners. The toxicity of a toxic substance is rated in relation to the compound with the greatest toxicity, 2,3,7,8-TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin). First, the concentration of this compound is multiplied by the relevant toxic equivalency factor (TEF) to give the TEQ value. The sum of TEQs for the individual toxic substances provides the total concentration of toxic equivalents, related to the effect of the concentration of pure 2,3,7,8-TCDD. WHO-PCDD/F-TEQ is the sum of toxicity equivalents of the 17 toxicologically most important polychlorinated dioxins and furans. The WHO-PCB-TEQ is the sum of toxicity equivalents of the 12 DL-PCBs that have been assigned TEFs, such as the dioxins to classify the toxicity of these PCB congeners relative to 2,3,7,8-TCDD. The sum of WHO-PCDD/F-TEQ and WHO-PCB-TEQ is called the total dioxin equivalent (WHO-PCDD/F-PCB-TEQ). The TEQ values thus represent toxic effects of a large group of toxic compounds, which greatly simplifies the evaluation of toxicological risks.

Determination of dioxin-like compounds utilising *in vitro* bioassays of induced enzymes ethoxyresorufin-*O*-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) are now used in many laboratories to quantify global PCDD, PCDF and PCBs toxicity levels. In the case of PCBs, only some congeners induce AHH, but NDL-PCB congeners that do not have the toxicity of dioxins also have their inherent toxicity. For example, some high-chlorinated mixtures of congeners with low TEF are responsible for the formation of liver tumours observed under certain circumstances (at high doses) in animals. New data on the occurrence of high concentrations of DL-PCBs in farmed fish (especially salmon) and the associated risks to consumers were impulses for the EFSA discussion in 2004, when it was found that these substances should be regulated along with dioxins. In 2006, DL-PCBs were included to the maximum value of the TEQ.

12.4.1.5.2 Toxic metabolites

Some hydroxy derivatives of PCBs can, through structural relationship with thyroxine, interfere with the metabolism of thyroid hormones by competitive inhibition of transthyretin (the serum and cerebrospinal fluid carrier of the thyroid hormone thyroxine and retinol binding protein) and hormonal processes in the body. Toxicological studies have confirmed that sulfur metabolites of PCBs may bind to the secretoglobin called uteroglobin (progesterone-binding protein). Arylmethylsulfones accumulate mainly in the lungs and probably play an important role in the aetiology of toxic manifestations of Yushō disease (Table 12.37). Although the toxicity of a wide range of chemicals depends on their biotransformation mediated by monooxidases (or other enzyme systems) to more toxic products, it has been demonstrated that the toxicity of PCBs is not dependent on their metabolic activation. The parent compounds and not their metabolites are therefore responsible for the toxic effects associated with exposure to PCBs.

12.4.1.6 Mitigation

For the active reduction of dibenzodioxins, dibenzofurans and PCBs in foods and feeds, European Commission Recommendation 2006/88/EC has been issued. With respect to risk management evaluation, so-called action levels have been published for dioxins and dibenzofurans and dioxin-like PCBs (WHO-TEQ) in foods and feeds, which may be used by control authorities and manufacturers to decide when it is appropriate to identify a source of contamination and to take measures for its reduction or elimination.

Commision Recommendation No. 1881/2006/EU lists maximum levels for WHO-PCDD/PCDF-TEQ and WHO-PCDD/PCDF-PCB-TEQ. If dioxine-like PCBs are not included

Table 12.37 Comparison of toxic equivalency factors (TEFs) for PCB congeners (for humans and mammals in general).

Congener	Structural type	TEF-WHO	Congener	Structural type	TEF-WHO
77	Non-*ortho*	0.0001[a]	118	Mono-*ortho*	0.000 03[d]
81	Non-*ortho*	0.0003[b]	123	Mono-*ortho*	0.000 03[d]
126	Non-*ortho*	0.1[a]	156	Mono-*ortho*	0.000 03[e]
169	Non-*ortho*	0.03[c]	157	Mono-*ortho*	0.000 03[e]
105	Mono-*ortho*	0.000 03[d]	167	Mono-*ortho*	0.000 03[f]
114	Mono-*ortho*	0.000 03[e]	189	Mono-*ortho*	0.000 03[d]

[a]The same in 1998.
[b]0.0001 in 1998.
[c]0.01 in 1998.
[d]0.0001 in 1998.
[e]0.0005 in 1998.
[f]0.000 01 in 1998.

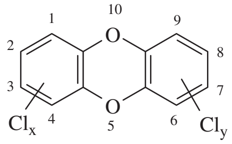
in the sum, the allowed values are approximately half the WHO-PCDD/PCDF-TEQ values. For example, for raw milk and dairy products, (including butterfat, hens' eggs and egg products) 3 pg/g fat of WHO-PCDD/PCDF-TEQ and 6 pg/g fat of WHO-PCDD/PCDF-PCB-TEQ are allowed. The highest values are set for meat and meat products (excluding edible offal) of bovine animals and sheep (3 and 4.5 pg/g fat), while lower values are set for poultry and pigs (2 and 4 pg/g fat and 1 and 1.5 pg/g fat, respectively). Higher values are set for muscle meat of fish and fishery products (4 and 8 pg/g wet weight). The highest values are allowed for liver of terrestrial animals (12 pg/g) and (by Commission Regulation No. 565/2008/EU) also for fish liver and its products (25 pg/g), which is close to the real concentrations in these products.

12.4.2 Polychlorinated dibenzo-*p*-dioxins and dibenzofurans

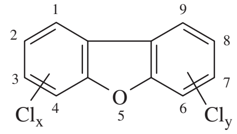
Polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) are, from the toxicological point of view, one of the most important groups of global environmental contaminants. Unlike all other halogen compounds, PCDD and PCDF do not have and have never had any practical use, and thus were never specifically synthesised in large quantities. They thus represent the category of unintentionally produced POPs (Table 12.26). The levles of these POPs that accumulate in food chains and are associated with health risks for living organisms (even in long-term exposure to very low concentrations) are the subject of special attention from experts, politicians and the public.

12.4.2.1 Structure and nomenclature

PCDD (**12-131**) and PCDF (**12-132**) are almost planar tricyclic compounds. Theoretically, the total number of PCDD derivatives is 75 (Table 12.38) and the total number of PCDF derivatives, which contain one to eight chlorine atoms, is 135 (Table 12.39).



12-131, polychlorinated dibenzo-*p*-dioxins



12-132, polychlorinated dibenzofurans

12.4.2.2 Properties

Properties of PCDD and PCDF are similar to other POPs, whose aromatic rings are substituted with halogens.

12.4.2.2.1 Physical and chemical properties

PCDD and PCDF are characterised by relatively high lipophilicity, high values of log K_{OW} and very low solubility in water. The characteristics of individual congeners can be found in specialised literature. Table 12.40 gives examples of some of the data for groups of high-chlorinated isomers of dibenzodioxins and for the perchlorinated congener octaCDD.

12.4.2.2.2 Persistence in living organisms

High-chlorinated PCDD and PCDF are generally characterised by considerable bioaccumulation potential and high persistence. Their exclusion from the exposed organism is very slow. Typical ranges of half-life values for elimination of toxic congeners from human

Table 12.38 Nomenclature of PCDD according to International Union of Pure and Applied Chemistry (IUPAC).

Number	Structure	Number	Structure	Number	Structure	Number	Structure
MonoCDD		19	1,3,6	39	1,2,7,8		
1	1	20	1,3,7	40	1,2,7,9	59	1,2,4,6,9
2	2	21	1,3,8	41	1,2,8,9	60	1,2,4,7,8
DiCDD		22	1,4,6	42	1,3,6,8	61	1,2,4,7,9
3	1,2	23	1,4,7	43	1,3,6,9	62	1,2,4,8,9
4	1,3	24	1,4,8	44	1,3,7,8	**HexaCDD**	
5	1,4	25	1,7,8	45	1,3,7,9	63	1,2,3,4,6,7
6	1,6	26	2,3,7	46	1,4,6,9	64	1,2,3,4,6,8
7	1,7	**TetraCDD**		47	1,4,7,8	65	1,2,3,4,6,9
8	1,8	27	1,2,3,4	***48***	***2,3,7,8***	***66***	***1,2,3,4,7,8***
9	1,9	28	1,2,3,6	**PentaCDD**		***67***	***1,2,3,6,7,8***
10	2,3	29	1,2,3,7	49	1,2,3,4,6	68	1,2,3,6,7,9
11	2,7	30	1,2,3,8	50	1,2,3,4,7	69	1,2,3,6,8,9
12	2,8	31	1,2,3,9	51	1,2,3,6,7	***70***	***1,2,3,7,8,9***
TriCDD		32	1,2,4,6	52	1,2,3,6,8	71	1,2,4,3,7,9
13	1,2,3	33	1,2,4,7	53	1,2,3,6,9	72	1,2,4,6,8,9
14	1,2,4	34	1,2,4,8	***54***	***1,2,3,7,8***	**HeptaCDD**	
15	1,2,6	35	1,2,4,9	55	1,2,3,7,9	***73***	***1,2,3,4,6,7,8***
16	1,2,7	36	1,2,6,7	56	1,2,3,8,9	74	1,2,3,4,6,7,9
17	1,2,8	37	1,2,6,8	57	1,2,4,6,7	**OctaCDD**	
18	1,2,9	38	1,2,6,9	58	1,2,4,6,8	***75***	***1,2,3,4,6,7,8,9***

adipose tissue are summarised in Table 12.41. For comparison, data for the three most toxic DL-PCB congeners are also presented.

12.4.2.3 Occurrence and main sources

PCDD and PCDF arise, unlike most other organochlorine contaminants, almost exclusively as byproducts of anthropogenic activities. Recent research has also confirmed the formation of trace amounts of PCDD and PCDF in enzymatically catalysed reactions. For example, they may be biosynthesised by microbial peroxidases in sewage sludge contaminated with chlorophenols. The most important primary emission sources were or still are:

- industrial syntheses or technologies, such as manufacture of certain pesticides (particularly hexachlorobenzene, pentachlorophenol or phenoxyalkanoic acids), production of technical mixtures of PCBs, production of intermediates of some organic products (especially chlorophenols and chlorobenzenes, but also other organic and inorganic chlorine compounds), bleaching of wood pulp with chlorine dioxide (ClO_2), production of chlorine using graphite electrodes;
- incineration of the above mentioned organic and inorganic compounds containing chlorine, such as waste incineration (municipal or paramedical), disposal of toxic compounds, metallurgy, fires involving burning of organic compounds containing chlorine (such as PVC), but the formation of dioxins can occur also in the presence of a chlorine donor during pyrolysis of unchlorinated materials such as polystyrene, cellulose, lignin or coal;
- photochemical reactions in the atmosphere and reactions of components found in emissions.

In addition to these primary sources, the contamination of the food chain may create various waste products, such as industrial compost, landfill materials, sewage sludges, contaminated industrial products, for example herbicides based on 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), technical PCB mixtures, biocide pentachlorophenol (PCP) and its salts.

Acquired knowledge of the mechanism of reactions leading to the formation of PCDD and PCDF and factors influencing their formation, along with the mapping of pathways of these pollutants

Table 12.39 Nomenclature of PCDF according to International Union of Pure and Applied Chemistry (IUPAC).

Number	Structure	Number	Structure	Number	Structure	Number	Structure
MonoCDF		34	1,4,8	69	1,3,6,8	104	1,2,6,7,9
1	1	35	1,4,9	70	1,3,6,9	105	1,3,4,6,7
2	2	36	1,6,7	71	1,3,7,8	106	1,3,4,6,8
3	3	37	1,6,8	72	1,3,7,9	107	1,3,4,6,9
4	4	38	1,7,8	73	1,4,6,7	108	1,3,4,7,8
DiCDF		39	2,3,4	74	1,4,6,8	109	1,3,4,7,9
5	1,2	40	2,3,6	75	1,4,6,9	110	1,3,6,7,8
6	1,3	41	2,3,7	76	1,4,7,8	111	1,4,6,7,8
7	1,4	42	2,3,8	77	1,6,7,8	112	2,3,4,6,7
8	1,6	43	2,4,6	78	2,3,4,6	113	2,3,4,6,8
9	1,7	44	2,4,7	79	2,3,4,7	***114***	***2,3,4,7,8***
10	1,8	45	2,4,8	80	2,3,4,8	**HexaCDF**	
11	1,9	46	2,6,7	81	2,3,6,7	115	1,2,3,4,6,7
12	2,3	47	3,4,6	82	2,3,6,8	116	1,2,3,4,6,8
13	2,4	48	3,4,7	***83***	***2,3,7,8***	117	1,2,3,4,6,9
14	2,6	**TetraCDF**		84	2,4,6,7	***118***	***1,2,3,4,7,8***
15	2,7	49	1,2,3,4	85	2,4,6,8	119	1,2,3,4,7,9
16	2,8	50	1,2,3,6	86	3,4,6,7	120	1,2,3,4,8,9
17	3,4	51	1,2,3,7	**PentaCDF**		***121***	***1,2,3,6,7,8***
18	3,6	52	1,2,3,8	87	1,2,3,4,6	122	1,2,3,6,7,9
19	3,7	53	1,2,3,9	88	1,2,3,4,7	123	1,2,3,6,8,9
20	4,6	54	1,2,4,6	89	1,2,3,4,8	***124***	***1,2,3,7,8,9***
TriCDF		55	1,2,4,7	90	1,2,3,4,9	125	1,2,4,6,7,8
21	1,2,3	56	1,2,4,8	91	1,2,3,6,7	126	1,2,4,6,7,9
22	1,2,4	57	1,2,4,9	92	1,2,3,6,8	127	1,2,4,6,8,9
23	1,2,6	58	1,2,6,7	93	1,2,3,6,9	128	1,3,4,6,7,8
24	1,2,7	59	1,2,6,8	***94***	***1,2,3,7,8***	129	1,3,4,6,7,9
25	1,2,8	60	1,2,6,9	95	1,2,3,7,9	***130***	***2,3,4,6,7,8***
26	1,2,9	61	1,2,7,8	96	1,2,3,8,9	**HeptaCDF**	
27	1,3,4	62	1,2,7,9	97	1,2,4,6,7	***131***	***1,2,3,4,6,7,8***
28	1,3,6	63	1,2,8,9	98	1,2,4,6,8	132	1,2,3,4,6,7,9
29	1,3,7	64	1,3,4,6	99	1,2,4,6,9	133	1,2,3,4,6,8,9
30	1,3,8	65	1,3,4,7	100	1,2,4,7,8	***134***	***1,2,3,4,7,8,9***
31	1,3,9	66	1,3,4,8	101	1,2,4,7,9	**OctaCDF**	
32	1,4,6	67	1,3,4,9	102	1,2,4,8,9	***135***	***1,2,3,4,6,7,8,9***
33	1,4,7	68	1,3,6,7	103	1,2,6,7,8		

Table 12.40 Physical and chemical properties of selected PCDD isomers.

Molecular formula (group of isomers)	Trivial name	Number of isomers	log K_{OW}	Solubility in water at 25 °C (mg/l)
$C_{12}H_4O_2Cl_4$	TetraCDD	22	6.9	0.000 35
$C_{12}H_3O_2Cl_5$	PentaCDD	14	7.4	0.000 12
$C_{12}H_2O_2Cl_6$	HexaCDD	10	7.8	0.000 44
$C_{12}HO_2Cl_7$	HeptaCDD	2	8.0	0.000 24
$C_{12}O_2Cl_8$	OctaCDD	1	8.2	0.000 40

Table 12.41 Elimination of selected PCDD, PCDF and planar non-*ortho* PCBs from human adipose tissue.

Substances	Congener no.	Elimination half-life (years)	Substances	Congener no.	Elimination half-life (years)
PCDD	48	5.8–9.6		118	2.9–6.2
	54	8.6–15.7		121	3.5–6.2
	66	8.4–19.0		124	3.8–5.9
	67	3.5– > 70		130	2.4–5.8
	70	4.9–8.5		131	2.6–6.5
	73	3.2–6.6		134	3.2
	75	5.6–6.7		135	<0.2
PCDF	83	0.4	Non-*ortho* PCB	77	0.1
	94	0.9		126	2.7
	114	4.7–19.6		169	13.0

into the environment, led to systematically applied restrictive measures in the related technologies as well as at the level of legislation. The result of the efforts to improve the situation at the turn of the last century is shown in Figure 12.49, from which a dramatic decrease of dioxin emissions from industrial sources is apparent, while emissions from transport or waste incineration and fires did not show such a downward trend.

12.4.2.4 Release into the environment

PCDD and PCDF can be released into the environment, and subsequently to the food chain, not only from these primary sources, but also from secondary sources, such as atmospheric deposition, point source emissions (various incinerators or industrial plants) and contaminated materials in the agroecosystem. For ruminants, the main route of dioxin intake is vegetation. For the aquatic ecosystem the main source of contamination is atmospheric deposition. In the initial phase, PCDD and PCDF (as well as other POPs) accumulate in the benthos, which is fed to animals at higher trophic levels. Often contamination of food sources as a result of violation of mandatory rules for their protection cannot be ruled out. Examples of problems that have occurred in developed European countries have included massive contamination of feed for livestock, which took place in Belgium in the late 1990s. In 1999, 500 tons of a contaminated batch of feed were distributed, which contained about 50 kg of PCBs and 1 g of dioxins. The feed was used in farms in Holland, Germany and France as well as Belgium. Another recent scandal involving contaminated feed for pigs and cattle occurred in Ireland in 2008. The profile of PCBs indicated penetration of transformer oil based on Aroclor. The overall contamination of chicks reached an average value of 170 ± 488 pg/kg for dioxins and 240 ± 2037 ng/kg for PCBs (TEQ in fat), and similarly high findings were discovered in eggs and milk. Contaminated meat was distributed to both the domestic and the foreign markets in the EU. Concentrations of PCDD/PCDF in pork and beef exceeded the safety limits by 80–200 times.

Source of dioxins in nearly 50 affected farms were old bakery products, which were dried by waste gases from contaminated fuel. Food and feed raw materials imported from developing countries may also be hazardous materials. In 2007, the Rapid Alert System for Food and Feed (RASFF) pointed out a high concentration of PCDD/PCDF (exceeding normal quantities by up to 1000 times) in guar gum found by control laboratories in Switzerland. The source of contamination was impurities in low-quality biocidal product

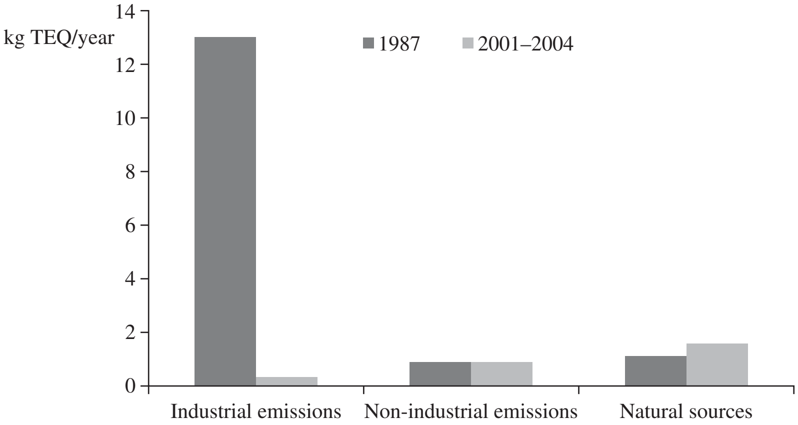


Figure 12.49 Changes in emissions of PCDD/PCDF (according to US Environmental Protection Agency, EPA: Trends in dioxin levels in the environment and in humans. Available at: http://heartland.org/sites/all/modules/custom/heartland_migration/files/pdfs/15201.pdf. Accessed: March 12, 2013.

based on pentachlorophenol (PCP) that has been in contact with the guar gum.

12.4.2.4.1 Occurrence in foods

Under normal circumstances, the concentrations of PCDD and PCDF in food raw materials and products are very low, usually in the range of units to tenths of μg/kg lipids, and only in exceptional cases, for example in fish from contaminated localities, reach values of an order of magnitude higher. Table 12.42 shows the levels of dioxins and DL-PCB in different foods on the Dutch market in 2004. With respect to similar toxic effects, concentrations (TEQ values) of these two types of contaminants are given together. In accordance with the previously mentioned facts, foods of animal origin contained higher concentrations of these contaminants than products of plant origin. This table also illustrates the relatively high contribution of DL-PCBs to the total TEQ value, which even exceeded the contribution of dioxins in some cases.

12.4.2.4.2 Human exposure

Human exposure to PCDD and PCDF occurs mainly via food. As with PCBs, it is expected that dietary intake represents up to 95% of the total amount of PCDD and PCDF. For the purpose of assessing health risks associated with exposure to these POPs, many countries have conducted studies to estimate their daily intake. Examples are given in Table 12.43. Attempts to find a correlation between the amount of PCDD and PCDF in a given area and their contents in human adipose tissue were generally unsuccessful, except in rural areas where a greater proportion of locally produced food is consumed. If an increased contamination is from a local source in these areas, higher levels

Table 12.42 Mean PCDD/PCDF and DL-PCB concentrations (TEQ, ng/kg).

Foods	Average fat content (%)	Dioxins	Non-*ortho*-PCB	Mono-*ortho*-PCB	Total TEQ[a]
Butter	81	0.96	0.52	0.03	1.50
Cheese	31	0.14	0.14	0.01	0.29
Eggs	10	0.09	0.03	0.003	0.12
Milk	1	0.01	0.01	0.001	0.01
Beef	16	0.05	0.13	0.001	0.18
Pork	26	0.09	0.02	0.004	0.11
Poultry meat	9	0.04	0.01	<0.001	0.05
Vegetable fats and oils	57	0.23	0.02	0.001	0.25
Cereals	1	0.09	<0.01	0.001	0.14
Fruits and fruit juices	-	0.13	0.01	0.001	0.15

[a]For the calculations, updated values of TEF from 2006 were used.

Table 12.43 Estimates of TCDD and PCDF income in different countries.

Country; year	Exposure TEQ,[a] population	Assumed concentration data	Main foods contributing at intake
Spain; 2002[b,c]	Mean–0.98; all population	Mean, only PCDD/PCDF	Fish (34%), fats (15%), cereals (14%), dairy products (14%)
Spain; 2000–2003	3.22	Upper limit of the estimate	Dairy products (29%), vegetable oils (19%)
Belgium; 1999	Mean–2.53; 14–18 years	Probability	Dairy products, fish, beef
Belgium; 2000–2001[b,c]	Mean-2.04	Lower limit, without *mono-ortho* PCB, includes wheat products and vegetables	Fish (40%), beef (35%), dairy products (27%)
Holland; 2004[d]	Mean–0.9; all population	Lower limit of the estimate	Dairy products (38%), vegetable oils (17%), meat (17%), fish (12%)
France; 2001–2004[b]	Mean–1.8; all population	Lower limit of the estimate, all foods	Fish (48%), dairy products (31%)
Italy; 2006[b]	Mean–2.28; 13–94 years	Upper limit of the estimate	Fish (44%), dairy products (27%)
Great Britain; 200[b]	0.5–0.9; 19–64 years	All foods	Meat, dairy products, fish
USA; 1999–2002[b]	–	All foods	Meat (32%), dairy products (16%), fish (39%)
Australia; 2000–2001[e,f]	Mean–3.0 (lower limit), 36.7 (upper limit); >2 years	Lower and upper limits of the estimate, only animal products, bakery products and peanut butter	Fish (39%), dairy products (31%), pork (7%)
Japan; 2004[b,g]	1.55	All foods (after heat treatment)	Fish (82%), meat, eggs (11%), dairy products (4%)

[a] In pg/kg body weight per day.
[b] Method of calculation: average consumption × average concentration.
[c] Body weight 65 kg.
[d] Long-term consumption × average concentration.
[e] Exposure expressed in pg/kg¯body weight per month.
[f] Method of calculation: consumption x concentration.
[g] Body weight 50 kg.

of PCDD/PCDF in human adipose tissue can be found. It is interesting to compare the data for Japan and the Netherlands. In countries with a high consumption of fish, like Japan, this commodity significantly contributes to the intake of dioxins and related POPs. In the Netherlands there is a relatively low consumption of fish, which is reflected in their small contribution to the total exposure. On the other hand, the significant contribution of milk and dairy products to the overall exposure documents the load of the terrestrial ecosystem by emissions from incinerators and other anthropogenic sources. Increased levels of PCDD/PCDF in milk and dairy products are alarming, because a large part of the population may be exposed via these commodities, including children, who are particularly vulnerable to the toxic effects of xenobiotics (chemical compounds that are foreign to living organisms).

In virtually all cases, the total TEF value is contributed to mostly by 2,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD, 1,2,3,6,7,8-hexaCDD, 2,3,4,7,8-pentaCDF and PCB 126. The contribution of DL-PCBs varies over a wide range and in some cases exceeds 50%. There is a high risk of increased exposure during lactation, as the gastrointestinal tracts of nursed babies absorb virtually all lipids contained in breast milk, and these lipids represent a reservoir of PCDD, PCDF and other POPs. PCDD/PSDF particularly predominate in breast milk in Belgium, the Netherlands, Luxembourg, Finland

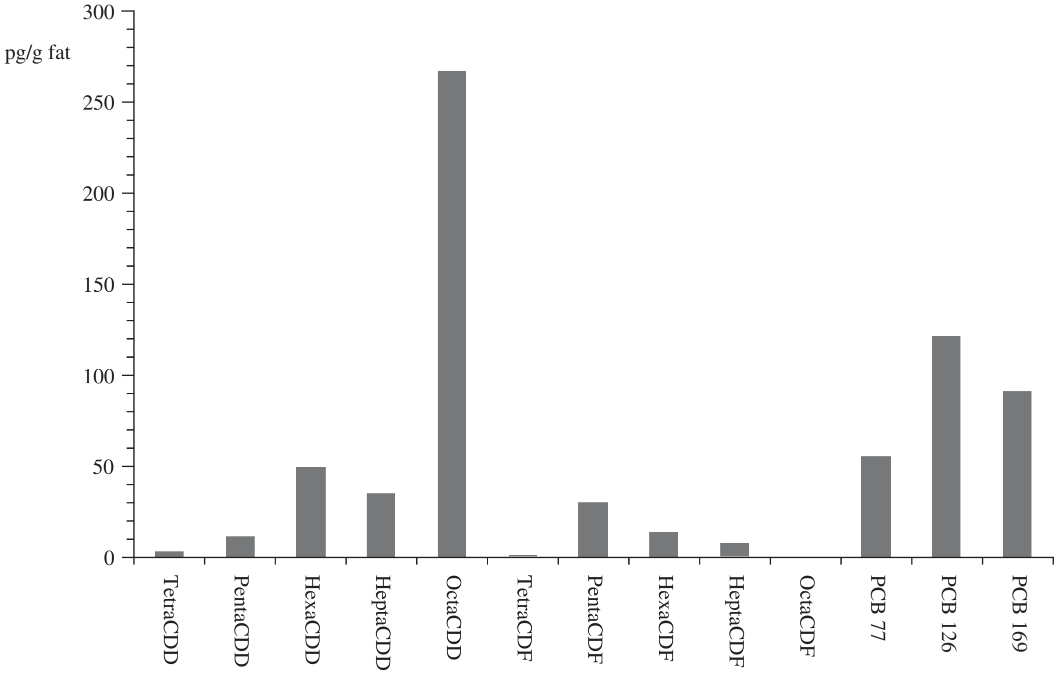


Figure 12.50 Profile of groups of PCDD/PCDF congeners and representatives of DL-PCBs in breast milk.

and Spain, but in some countries (e.g. in the Czech Republic or Ukraine) DL-PCBs significantly dominate. Figure 12.50 illustrates the variability of individual groups of PCDD/ PCDF and DL-PCBs in the milk of mothers that are breastfeeding their first child. In mothers nursing twins, the main breast milk components were hexaCDD, pentaCDF, hexaCDF and heptaCDF. The mobility of individual congeners (rate of their excretion) is not the same, but the total concentration of POPs during lactation decreases (in milk and generally in the organism of the mothers), unless there is a new dietary exposure. The authorities in some Baltic countries even issue recommendations during pregnancy and lactation to reduce consumption of fish from this area in view of their relatively high contamination, as the decrease in dietary intake of PCDD/PCDF is accompanied by decreases in plasma and breast milk.

12.4.2.5 Health and toxicological assessment

Many PCDD/PCDF congeners are characterised by considerable toxicity and their relative effect is related (as in DL-PCB) to congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which was selected on the basis of a series of *in vivo* and *in vitro* toxicological studies. The equivalent factor (TEF) of TCDD was set to one.

TCDD and related compounds have negative effects on several organs in different animal species. The most famous case where people were exposed to dioxins was the chemical plant accident in Seves in Italy that occurred in 1976. The most important harmful effects are immunotoxicity, behavioural disorders and reproductive disorders. Subchronic effects in experimental animals include weight loss, pathological changes in the liver, thymus, lymph glands, hair loss, porphyria (decomposition of haemoglobin) and some others. Evidence of the carcinogenicity of 2,3,7,8-tetraCDD is also ambiguous; the IARC classifies this substance as a Class 1A carcinogen.[3] TetraCDD and related compounds act at different levels, through initiation of hormonal mechanisms supporting carcinogenic processes, on the development of the intellect in the foetus just before birth or shortly after birth (doses received by breast milk may exceed the normal dietary intake of adults). As in the case of PCBs, both PCDD and PCDF (depending on the structure of the individual congeners and their planarity, respectively) induce a hepatic microsomal system of oxidases (cytochrome P450) in exposed organisms. The most effective inducer is the above mentioned TCDD. Values of toxic equivalency factors (TEFs) have been the subject of intensive technical discussions for many years. In 2005, an expert group of the WHO-IPCS (International Programme on Chemical Safety) confirmed the correctness of the concept based on the additivity of their effects, and reconsidered older TEF values in terms of new scientific knowledge. Table 12.44 shows the relative toxicity of 2,3,7,8-tetraCDD and other monitored PCDDs and PCDFs for humans and other mammals. TEF values, which were estimated for other species and used in assessing ecotoxicological risks, may be more or less different. A particularly significant difference in the intensity of biological responses and the TEF values is for fish.

12.4.2.6 Mitigation

Measures aimed at reducing emissions of PCDD/PCDF into the environment and contaminations of food chains have undoubtedly influenced the decline in population exposure in recent years. Interesting in this regard is a study of the dynamics of the levels of

Table 12.44 Comparison of TEF values for PCDD and PCDF congeners used to estimate the exposure risk.

Chlorinated dibenzo-*p*-dioxins	TEF-WHO	Chlorinated dibenzofurans	TEF-WHO
2,3,7,8-TetraCDD[a]	1	2,3,7,8-tetraCD[a]	0.1
1,2,3,7,8-PentaCDD[a]	1	1,2,3,7,8-pentaCDF[c]	0.03
1,2,3,4,7,8-HexaCDD[a]	0.1	2,3,4,7,8-pentaCDF[d]	0.3
1,2,3,6,7,8-HexaCDD[a]	0.1	1,2,3,4,7,8-hexaCDF[a]	0.1
1,2,3,7,8,9-HexaCDD[a]	0.1	1,2,3,6,7,8-hexaCDF[a]	0.1
1,2,3,4,6,7,8-HeptaCDD[a]	0.01	1,2,3,7,8,9-hexaCDF[a]	0.1
OctaCDD[b]	0.0003	2,3,4,6,7,8-hexaCDF[a]	0.1
		1,2,3,4,6,7,8-heptaCDF[a]	0.01
		1,2,3,4,7,8,9-heptaCDF[a]	0.01
		octaCDF[b]	0.0003

[a]The same in 1998.
[b]0.0001 in 1998.
[c]0.05 in 1998.
[d]0.5 in 1998.

PCDD/PCDF in the US population born between 1950 and 1980 (Figure 12.51). While in the oldest persons the levels of bioaccumulated pollutants were on average almost 15 ng/kg fat, people born in the 1980s were exposed to these POPs significantly less.

In 2000, the WHO calculated the TDI values in the range from 1 to 4 pg WHO-PCDD/PCDF-PCB-TEQ per kg body weight. These exposure limits are based on non-carcinogenic effects, with the main ones to be evaluated being immunotoxicity, neurotoxicity and interference with hormonal systems. In 2001, the Scientific Committee on Food (SCF) of the European Union (EU) calculated a tolerable weekly intake (TWI) of 14 pg WHO-PCDD/PCDF-PCB-TEQ per kg body weight. In the same year, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) calculated a provisional tolerable monthly intake (PTMI) of 70 pg WHO-PCDD/PCDF-PCB-TEQ per kg body weight and month. In 2006, the SCF confirmed the provisional tolerable weekly intake of 14 pg TEQ per kg body weight, which corresponds to the value of 2 pg TEQ per kg body weight per day proposed in 2001. Setting these limits causes serious consequences in the restrictive measures aimed at reducing exposure to PCDD/PCDF. Tolerable daily intake must always protect all populations and from this perspective the exposure of infants to breast milk is critical. Despite these facts, from the point of view of nutrition, breastfeeding is considered irreplaceable and so the only way to reduce this risk is by a drastic reduction in population exposure. The target value is 1 pg TEQ per kg of body weight per day. Under normal circumstances, the intake of PCDD/PCDF is much lower. The risks that could arise from ordinary low concentrations of these contaminants are not yet sufficiently documented, so it is recommended to reduce the intake of PCDD and PCDF to a minimum.

Examples of maximum levels set for the sum of dioxins (WHO-PCDD/PCDF-TEQ) and sum of dioxins and dioxin-like PCBs (WHO-PCDD/PCDF-PCB-TEQ) in foods are given in Section 12.4.1.6.

12.4.3 Other persistent organohalogenated contaminants

12.4.3.1 Brominated flame retardants

The development of anthropogenic activities is accompanied, among other things, by the growing use of combustible synthetic materials, which brings an increased risk of fire. One of the ways to prevent economic impacts and the possible loss of human lives is the use of flame retardants, chemicals that reduce the flammability of materials or delay their combustion. For example, many plastics are highly flammable and therefore their fire resistance is increased by adding flame retardants. These synthetic chemicals are used in electronics, upholstery, carpets, textiles, insulation, vehicle and airplane parts, baby blankets, children's clothes and strollers and many other products. Currently, nearly 180 different compounds that increase the fire safety of flammable materials are known. Taking into account their composition, flame retardants can be divided into four groups:

- inorganic compounds (salts)
- organophosphates
- organic compounds containing nitrogen
- halogenated compounds.

In terms of food safety, the most frequently monitored substances are halogenated compounds that are potentially dangerous to

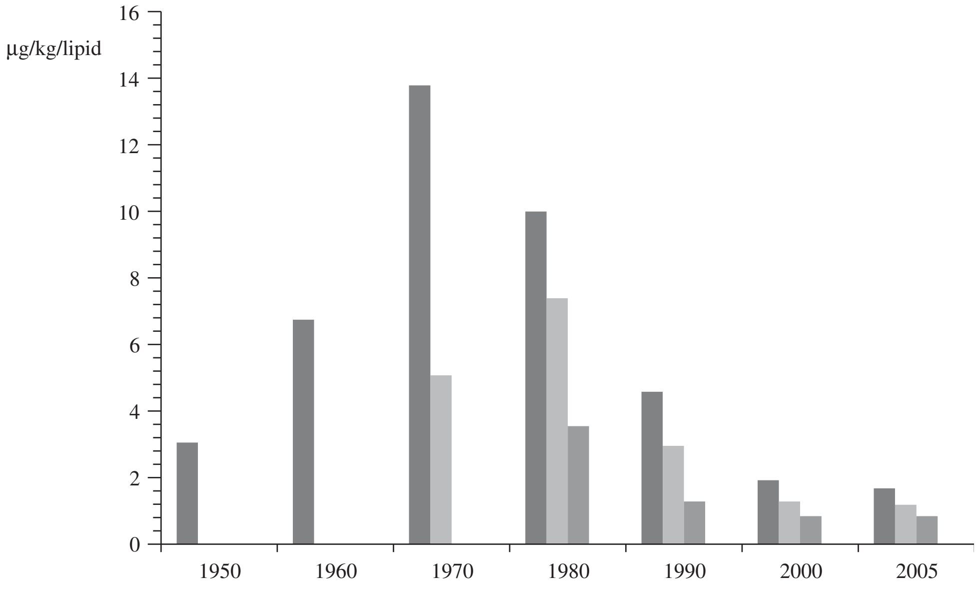
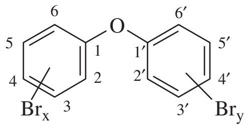


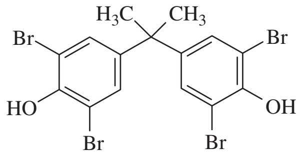
Figure 12.51 Average content of 2,3,7,8-TCDD (ng/kg) in the population of the United States born in 1950, 1970 and 1980.

the environment. The most important flame retardants of this group are brominated flame retardants (BFRs). The first mention of the occurrence of BFRs in the environment was in the late 1970s in Sweden when these substances were identified in fish. Greater attention began to be paid to BFRs during the 1990s when they were found in breast milk. Like PCBs and other persistent organochlorine compounds, residues of BFRs can be found in virtually all environmental components, biotic and abiotic, for example in the air, water, soil, sediments, sewage sludges, fish, birds and mammals, including humans. In addition to leaks during their production, their presence in the environment is related particularly to the use of materials containing flame retardants. An important source of BFRs is the disposal of plastics, electronic equipment and other wastes. These compounds can be leached from landfills, into the environment and, furthermore, polybrominated flame retardants release toxic polybrominated dioxins and furans when burned.

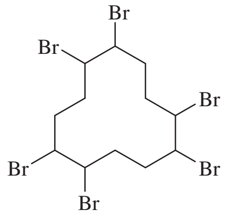
Today there are more than 75 different types of these substances, the classic examples of which include brominated biphenyls, diphenyl ethers, cyclododecane and bisphenol A. The use of individual flame retardants depends on the type of polymer, use of the product, its durability and the desired aesthetic appearance. The most commonly used BFRs are mixtures of polybrominated diphenyl ethers (PBDEs, **12-133**), 2,2′,6,6′-tetrabromobisphenol A (TBBPA, **12-134**) and 1,2,5,6,9,10-hexabromocyclododecane (HBCD or HBCDD, **12-135**), but the regular classic BFRs are still polybrominated biphenyls (PBB, **12-136**).



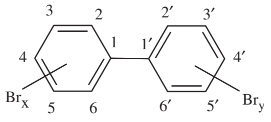
12-133, polybrominated diphenyl ethers



12-134, 2,2´,6,6´-tetrabromobisphenol A



12-135, 1,2,5,6,9,10-hexabromocyclododecane



12-136, polybrominated biphenyls

Table 12.45 shows the total consumption of BFRs in 2001. It is interesting to note the very different use of BFRs in different parts of the world, which naturally corresponds to the different natures of the contaminations. Following the development of the consumption of BFR in recent decades in Sweden (45 tons of PBDEs, 275 tons of TBBPA and 40 tons of HBCD in 1998 and 4 tons of PBDEs, 30 tons of TBBPA and 3 tons of HBCD in 2007) it may appear that the use of BFRs is declining, but in fact it is not, as the types of flame retardants in the market are constantly changing, mainly as a result of changes in legislation (including bans on the

Table 12.45 Consumption of the main types of BFRs on continents in tons.

Compound	Europe	Northern America	Asia	Others	Total
TBBPA	11 600	18 000	89 400	600	11 9600 (59%)
DecaBDE	7600	24 500	23 000	1050	56 150 (28%)
HBCD	9500	2800	3900	500	16 700 (8%)
PentaBDE	150	7100	150	100	7500 (4%)
OctaBDE	610	1500	1500	180	3790 (2%)
Total	29 460 (14.5%)	53 900 (26.5%)	11 7950 (57.5%)	2430 (1.2%)	203 740 (100%)

use of certain conventional compounds or their groups). It is very difficult to obtain current information about the production and use of new BFRs, because it is (from the perspective of the industry) sensitive data.

The mechanisms of the actions of flame retardants differs between the groups. In the case of halogenated compounds, the burning process is slowed by removing hydrogen and hydroxyl radicals from the flame. At higher temperatures flame retardants release free bromine or chlorine radicals, which react with hydrocarbons and yield hydrogen bromide or hydrogen chloride that react with combustion products to form water and bromine or chlorine radicals.

According to the method of incorporation into polymers, flame retardants can be divided into two basic groups:

- reactive
- additive.

Reactive flame retardants are added to the monomers, therefore they are covalently linked in the polymer and do not significantly penetrate into the environment. The use of these substances is limited to a few groups of polymers. Additive flame retardants are added into polymers, therefore they are not covalently bound, which is associated with the higher risk of environmental contamination. PBDEs and HBCD are used only as additive flame retardants, while TBBPA is primarily a reactive flame retardant and in only about 10% of cases is it used as an additive flame retardant. For example, the HBCD content in polystyrene foam ranges from 0.4 to 8% and the TBBPA content in polyesters may reach 13–28%.

12.4.3.1.1 Polybrominated diphenyl ethers

Structure and nomenclature

Polybrominated diphenyl ethers (PBDEs, **12-133**) are compounds structurally similar to polychlorinated biphenyls (PCBs). The numbering of 209 individual PBDE congeners is analogous to the IUPAC nomenclature used for numbering PCBs (Figure 12.46). The basic skeleton is diphenyl ether with a different number and location of bromine atoms. 209 possible congeners can be derived, which include 3 mono-, 12 di-, 24 tri-, 42 tetra-, 46 penta-, 42 hexa-, 24 hepta-, 12 octa-, 3 nona- and 1 decabromodiphenylether isomers.

Production

PBDEs are synthesised industrially mainly by bromination of diphenyl ether, which produces a mixture of different compounds. Not all congeners are or have been commercially used. Four technical PBDE mixtures were applied in the industrial production: decaBDE (decabromodiphenyl ether), octaBDE, pentaBDE and tetraBDE. DecaBDE contains about 97% of 209 BDE congeners and 3% of nonaBDE. A technical octaBDE mixture contains mainly heptaBDE and octaBDE, but to a lesser extent hexa-, nona- and decaBDE. The technical mixture pentaBDE contains predominantly penta- and tetrabromodiphenyl ether congeners. The technical mixture tetraBDE, containing tetra-, penta-, hexa- and partly other unidentified PBDEs, has a limited use. Industrial production in the EU and the US has been focused mainly on mixtures containing pentaBDE, octaBDE and decaBDE. The typical composition of PBDEs sold under the trademark Broukal is given in Table 12.46.

Properties

Physical and chemical properties PBDEs are very stable compounds, having a boiling point in the range of 310–425 °C, low volatility, high lipophilicity and low solubility in water (log K_{OW} is in the range of 4–10), which decreases with increasing number of bromine atoms. They are very persistent compounds, having a tendency to accumulate in different parts of the environment. Most studies show that low-brominated congeners are more persistent than the high-brominated congeners, which easily undergo degradation under bromine release. UV light significantly accelerates debromination (the most labile compounds are decaBDEs). It is therefore difficult to balance the contribution of individual technical mixtures of PBDEs to the contamination of environmental components, as these mixtures contain the originally present compounds together with products of photolysis of high-brominated PBDEs. A summary of selected chemical and physical properties of PBDE mixtures is shown in Table 12.47. A brief overview of the use of technical mixtures of PBDEs as flame retardants in

Table 12.46 Composition of technical mixtures of PBDEs (% w/w).

PBDE	Group of substances						
	TetraBDE	PentaBDE	HexaBDE	HeptaBDE	OctaBDE	NonaBDE	DecaBDE
TetraBDE	41	44-5	6-7	-	-	-	-
PentaBDE	24-38	50-60	4-8	-	-	-	-
OctaBDE	-	-	10-12	44	31-35	10-11	<1
DecaBDE	-	-	-	-	-	<3	97-98

Table 12.47 Selected physical and chemical properties of individual groups of polybrominated diphenyl esters.

Properties	TetraBDE	PentaBDE	OctaBDE	DecaBDE
Molecular formula	$C_{12}H_6Br_4O$	$C_{12}H_5Br_5O$	$C_{12}H_2Br_8O$	$C_{12}Br_{10}O$
Relative molecular weight	485.8	564.8	801.5	959.2
Major congeners	47, 49	66, 85, 99, 100	-	209
Melting point (°C)	80-200	−7 to −3	200	290-306
Decomposition point (°C)	-	> 200		> 320
Saturated vapour pressure (mPa)	-	1.23 (22 °C)	$<10^{-8}$ (25 °C)	$<10^{-8}$ (20 °C)
Solubility in water (mg/l)	-	9.10^{-7} (20 °C)	<0.01 (25 °C)	0.02-0.03 (25 °C)
log K_{OW}	5.87-6.16	6.64-6.97	8.35-8.90	9.97

various materials, which can be a source of emissions, is given in Table 12.48.

The most important congeners, which are monitored in the environment, include congeners PBDE 28 (2,4,4′-tribromodiphenyl ether, PBDE 47 (2,2′,4,4′-tetrabromodiphenyl ether), PBDE 99 (2,2′,4,4′,5-pentabromodiphenyl ether), PBDE 100 (2,2′,4,4′,6′-pentabromo diphenylether), PBDE 153 (2,2′,3,4, 4′,5′-hexabromodiphenyl ether), PBDE 154 (2,2′,4,4′,5,6′-hexabromodiphenyl ether) and PBDE 209 (2,2′,3,3′,4,4′,5,5′,6,6′-octabromodiphenyl ether).

Persistence in living organisms The majority of PBDEs are a group of highly lipophilic compounds, which are almost insoluble in water and can therefore accumulate in living organisms by bioaccumulation and biomagnification processes. The bioaccumulation potential of low-brominated congeners is very high (BCF value is higher than 5000).

Occurrence, main sources and release into the environment

Concentrations of PBDE congeners and their amount in the various components of the environment depend essentially on their structure (physico-chemical properties). The most persistent low-brominated congeners (with 4–7 Br atoms) may be found dissolved in water and also in the air, and therefore are transported over long distances. Their behaviour in the environment is similar to the behaviour of chlorinated POPs, such as PCBs or PCDD/PCDF. The majority of PBDEs used are high-brominated congeners, which are (due to their low volatility, low water solubility, lower bioaccumulation and high degree of adsorption on solid particles) less mobile in the environment and are found mostly in sediments, sludges and dust in the vicinity of emission sources. The composition of commercial mixtures replicates the representation of individual PBDEs in abiotic environments (such as sediments). Penta-, octa- and decaBDE congeners are therefore particularly predominant, the latter of which predominates only if photolysis does not occur. The spectrum of PBDEs in living organisms is sometimes fundamentally different due to the selective transfer from food or different biomagnifications.

Contamination of aquatic ecosystem, sewage sludge and soil

Low-brominated PBDE congeners are relatively soluble in water and their concentrations in sediments and soils are lower than the concentrations of high-brominated congeners that are strongly bound to particles in water, sediments and soils, especially to the particles with high organic carbon contents. Sediments, especially sediments in the vicinity of plants using brominated flame retardants, contain increased amounts of these contaminants. The representation of the individual substances in river sediments can vary significantly along the river, due to the presence of a range of local sources of pollution. The dominant congener is often

Table 12.48 Overview of some applications of PBDEs used as flame retardants in polymers.

Type of polymer or resin	Area of application
Acrylonitrile-butadiene-styrene	TVs, computers, hair dryers
Epoxy resin	Computers, electronic components
Phenolic resin	Paper laminates
Polyacrylonitrile	Electronic equipment, light panels
Polyamide	Computers, electronic connectors, garages
Polybutylene	Switches, fuses
Polyethylene	Electrical wiring, insulation materials
Polyethylene terephthalate	TVs, electronic equipment
Polypropylene	TVs, electronics
Polystyrene	Smoke detectors, office equipment, electronics covers
Polyvinyl chloride	Wires, cables, floor coverings
Polyurethane	Furniture, sound insulation, wood imitation
Textiles	Carpets, car interiors, furniture, tents, military safety clothing
Rubber	Conveyor belts
Coatings	Industrial paints, such as paints for protection of container

BDE 209, which can be present in concentrations amounting to 100–1000 μg/kg of dry sediment. Also present are lower congeners, such as BDE 47, BDE 99, BDE 100, BDE 153, BDE 154 and BDE 183 (in tenths, not more than tens, μg/kg dry sediment).

Sewage sludge from waste water treatment plants is considered one of the greatest sources of various contaminants that are only degradable with difficulty, including PBDEs. In many countries, sewage sludge is still used in agriculture for soil enrichment by organic components, which contributes to a substantial increase in the soil contamination (often to thousands of μg/kg dry matter), and consequently to the load of the food chain by PBDE and other undesirable chemicals. Often present in high concentrations is congener BDE 209, TBBPA and HBCD (100–1000 μg/kg dry matter). Information on these substances, however, is limited because frequently they are not the target analytes as are congeners BDE 47, BDE 99, BDE 100, BDE 154 and BDE 153, although they occur in smaller amounts. Representation of these congeners in the abiotic components of the environment replicates the composition of technical mixtures of pentaBDE, where the main component is again BDE 209. Elevated levels of soil contamination are also observed in the site of a conflagration.

Contamination of animals

Owing to the ubiquity and persistence in the environment of brominated flame retardants, it is not surprising that these substances penetrate into the tissues of animals, including humans, where they accumulate. As with other halogenated POPs, their concentration increases with each trophic level in a food chain. A higher range of biomagnification of brominated PBDEs indicates their easier absorption in the body. Concentrations of brominated flame retardators in organisms range from μg/kg fat to mg/kg fat, depending on the dietary habits of the species, age and level of contamination. The highest concentrations are found in the tissues of predatory fish.

The relative representation of individual PBDE congeners may be somewhat different interspecies. However, the dominant PBDE is often tetraBDE 47, which may constitute more than 50% of the total amount of PBDEs. Other relatively frequently occurring PBDEs are congeners 99, 100, 153 and 154. Despite the extensive use and common occurrence in the environment, the amounts of BDE 209 are relatively low. Previously, it was assumed that it is due to the large effective volume of its molecule, which cannot pass through biomembranes and thus accumulates in the tissues of living organisms. However, recent research revealed the presence of BDE 209 in the eggs of raptors (20–430 μg/kg fat) and fish (about 50 μg/kg fat), which clearly refutes the initial hypothesis and suggests instead a relatively rapid elimination of this perbrominated congener from the environment, even before its accumulation occurs. The finding of BDE 209 in the tissues of animals thus indicates the actual exposure of the organism to this type of technical mixtures.

Human exposure

Human exposure to brominated flame retardants occurs in various ways, and a human organism can be exposed to these contaminants and other halogenated compounds even before birth, as they may cross the placenta. Subsequently, the main exposure of infants is dietary exposure through breast milk, and later via the consumption of foods of animal origin with a higher fat content, especially contaminated fish and seafood.

In breast milk the main congener is BDE 47 (32–63%), while other majority congeners are BDE 99, BDE 100, BDE 153 and BDE 154 (minor differences in the spectrum of PBDEs in breast milk samples collected in different parts of the world appear to be linked to the different compositions of technical mixtures of brominated flame retardants). The total content of these PBDE congeners is highest in developed countries. For example, in the United States in 2003, their content was about 7 μg/kg fat, which corresponds to high concentrations of brominated flame retardants in virtually all parts of the environment. It could be expected that the adopted restrictive measures will be reflected in a decrease in the amount of PBDEs in the human environment, which should be associated with a decrease in their concentration in breast milk. In human tissues (as well as in other animal species) congeners BDE 47, 99, 100, 153 and 183 occur in the largest quantities. The subcutaneous fat contains in most cases the dominant congener BDE 153, which is followed

by congener BDE 47. The cause of the different distributions of individual congeners may be due to varying intakes of PBDEs from the air, where high-brominated congeners occur, especially in higher dust concentrations. Different kinetics of accumulation and biotransformation of individual congeners in the body may also play a role. The highest amounts of PBDEs were found in the plasma of workers who dismantled electronic products containing brominated flame retardants.

An important source of human exposure to brominated flame retardants is dietary intake, particularly the consumption of contaminated fish, as well as other foods with a higher fat content, such as meat, eggs, dairy products, fats and oils. Results obtained in Spain in 2000 are summarised in Table 12.49 as an example. The total daily intake of PBDEs was estimated to be 82–97 ng, including intake from fish and seafood at the level of 30 ng, from vegetable oils 24 ng and from meat and meat products 20 ng, which corresponds to a daily intake of 1.2–1.4 ng/kg body weight. Representation of individual congeners in food is consistent with their representation in biotic matrices, including human tissues. The dominant congeners are tetraBDE and pentaBDE. Variations in different countries are mainly caused by differences in the consumer's food basket. For example, in Sweden, the main congeners of the consumer's food basket (fish, meat, dairy products, eggs, fats and oils and bread) are BDE 47, 99, 100, 153 and 154, and the total daily intake of PBDEs is estimated at 51 ng.

Unlike for PCBs and other POPs, another important source of PBDEs is inhalation of contaminated dust or dermal intake. During recent years, much attention has focused on brominated flame retardants in dust, where high-brominated substances are mainly found, which have a higher affinity for solid particles. By far the highest concentration of these substances is found in indoor environments, due to a number of sources (electronics, furniture and various textiles). In the organism, these substances bind to blood lipids and are stored in adipose tissue. During lactation they are excreted to breast milk, and can be further metabolised to lower congeners or different, especially hydroxylated derivatives. The half-life of metabolic transformation of BDE 209 is a few days or weeks, and the half-life of congener 183 is 3 months. The most persistent congener is BDE 153.

Table 12.49 Content of PBDEs in selected foods.

Food	Content	
	ng/kg fat	ng/kg dry matter
Vegetables	-	5-8
Fruits	-	0-6
Cereals	-	0-36
Shellfish	2961-3140	83-88
White fish	2052-2359	37-88
Fish tins	1997-2117	246-260
Pork meat and products	565-597	166-172
Poultry meat	0-247	0-10
Beef and products	248-290	36-42
Eggs	482-530	58-64
Milk	525-630	20-24
Dairy products	557-677	34-48
Vegetable oils	795-805	794-804

In 2006, the EFSA's (European Food Safety Authority) panel on contaminants in the food chain (CONTAM) recommended member countries to monitor the following brominated flame retardants in food and feed: PBDE congeners 28, 47, 99, 100, 153, 154, 183 and 209, HBCD (sum of isomers) and PBB congener 153. Monitoring programmes could also include other PBDE congeners, decabromodiphenylethane (**12-143**), hexabromobenzene and bis(2,4,6-tribromophenoxy)ethane.

Biodegradation

To estimate the health risks represented by brominated flame retardants, it is important to consider, in addition to assessment of the potential routes of exposure, the biotransformation of these compounds in the body. The most abundant congener BDE 47 (2,2′,4,4′-tetrabromodiphenylether) occurring in biotic materials is absorbed up to 95% in the gastrointestinal tract of mice , distributed to the tissues and slowly metabolised. Only a small amount is excreted via excrements. Similarly, as in the case of aromatic xenobiotics, by the action of cytochrome, P450 complex epoxides are produced as the primary products, which, on hydrolysis, yield the corresponding hydroxy derivatives (Figure 12.52).

The metabolism of congener BDE 209 (2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether) is somewhat different. In comparison with PDA 47, BDE 209 is a substance with a significantly higher molecular weight, which reduces its absorption by the body to 10–25%. HydroxyoctaBDE, hydroxynonaBDE and hydroxymethoxyhexaBDEs were found in the blood plasma of rodents, and a large number of metabolites were also present in the rodents' faeces. Methoxylated and hydroxylated PBDEs have similarly been found in organisms of other mammals, including humans, the source of which could be fish, marine mammals or cyanobacteria, such as *Oscillatoria spongeliae*, living in symbiosis with marine sponges, algae, mussels and other seafood organisms. Debrominated, hydroxylated and methoxylated metabolites were also found in maize that had been exposed to low-brominated diphenyl ethers. The structures of some metabolites are given in formulae **12-137**.

Health and toxicological assessment

The acute toxicity of commercially used technical mixtures tested on laboratory rats is relatively low (LD_{50} <1 g/kg body weight), but at high doses increased liver weights and changes in the structure of liver tissue have been found. From a toxicological point of view, long-term exposure to low doses of PBDEs is a much greater risk. The evidence of the harmful effects of PBDEs includes interference with the endocrine system, (hormonal processes) as PBDEs

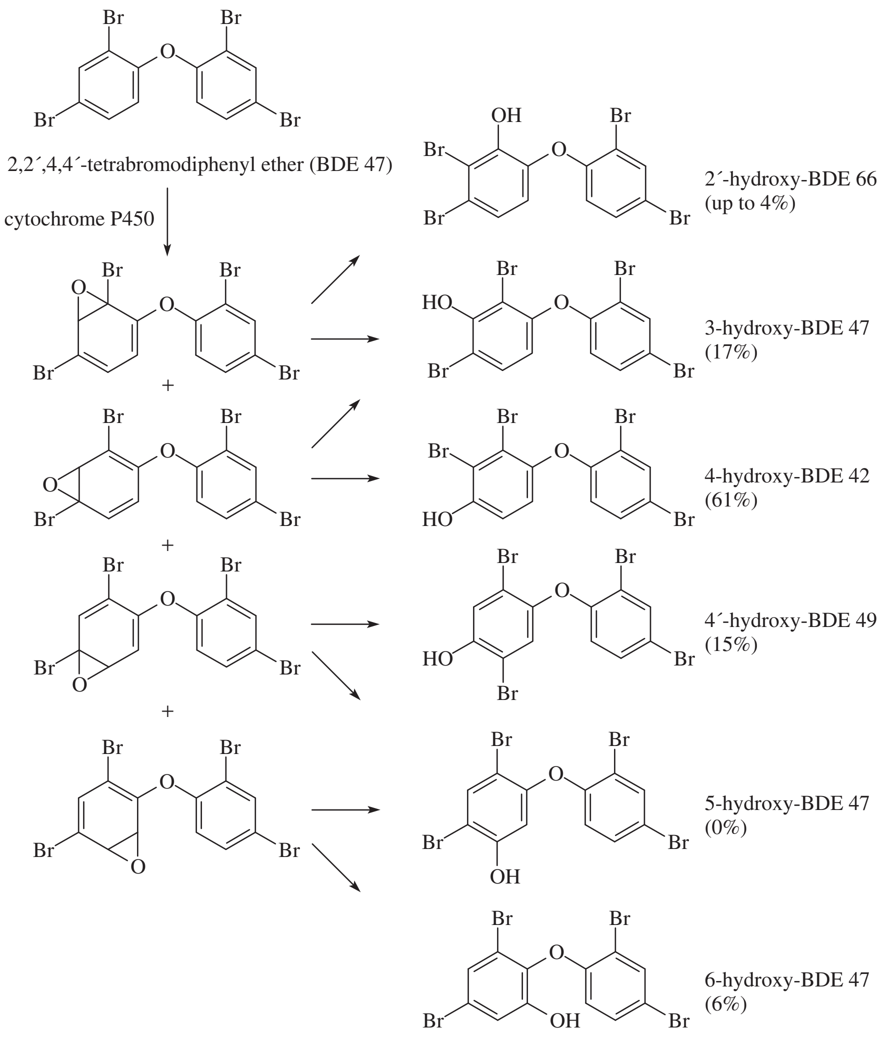
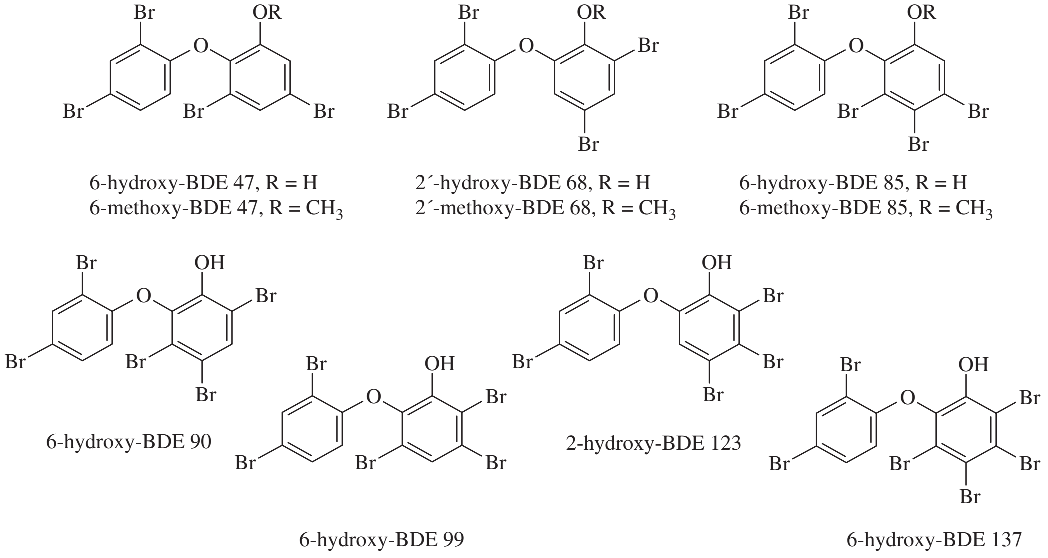
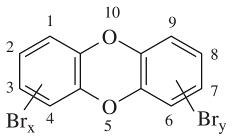


Figure 12.52 Metabolites of BDE 47.

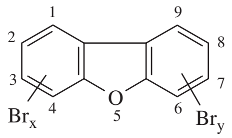


12-137, hydroxy and methoxyl derivatives of polybrominated diphenyl esters

act as endocrine disruptors. One of the most striking symptoms observed in animals was the increase of thyroxine concentration in the blood plasma, because some PBDEs (especially their hydroxyl derivatives) have a structure similar to thyroid hormones. Other possible adverse biological effects include neurotoxicity. In addition to the adverse biological effects induced by PBDEs, there also exists a risk of the formation of toxic substances during photolysis or pyrolysis of parent compounds. The most dangerous products are polybrominated dibenzo-*p*-dioxins (PBDD, **12-138**) and polybrominated dibenzofurans (PBDF, **12-139**).



12-138, polybrominated dibenzo-*p*-dioxins



12-139, polybrominated dibenzofurans

Mitigation

The European Union pushed for the elimination of brominated flame retardants in the 1990s. At present, there is a ban in the EU and US on the production of pentaBDE and octaBDE. Since 2009, the EU has also prohibited the use of decaBDE for electronic equipment. Even so, a large number of products containing these brominated flame retardants are still in use, which results in a continuous release of these substances into the environment.

12.4.3.1.2 Polybrominated biphenyls

Structure and nomenclature

Polybrominated biphenyls (PBBs, **12-136**) represent an older group of brominated flame retardants; 209 possible congeners of PBBs can be derived, but only 101 individual congeners have had registration numbers assigned.

Production PBBs are synthesised by the Friedel–Crafts reaction, in which biphenyl reacts with bromine in the presence of a catalyst ($AlCl_3$ or Fe). Commercial mixtures of PBBs, produced since the early 1970s, contained mainly hexa-, octa-, nona- and decabromobiphenyls.

Properties

Physical and chemical properties PBBs are solids that exhibit high chemical stability and resistance to acids, alkalis, oxidising and reducing agents, and are very thermostable. They are virtually non-volatile and insoluble in water; their solubility decreases with an increasing degree of bromination. Most PBBs have the value of log $K_{OW} > 7$ as they are highly lipophilic substances bioaccumulating in living organisms. A summary of selected chemical and physical properties of commercial mixtures of PBBs are presented in Table 12.50.

PBBs are primarily added as additive flame retardants, for example to acrylonitrile–butadiene–styrene polymers, which have found applications in the automotive industry, electronics and coatings. Since the 1970s, there has been stagnation in the production of PBBs, and the last known commercial production of decaBB was completed in France in 2000.

Occurrence and main sources

Data on the occurrence of PBBs in foods are essentially unavailable. The most serious penetration of these POPs into food chains occurred in the 1970s in Michigan, USA. Contamination of feed by technical mixtures FireMaster BP-6 led to high concentrations in a variety of foods of animal origin.

Health and toxicological assessment

At present, no comprehensive toxicological evaluation of PBBs is available. It can be assumed, however, that due to the structural

Table 12.50 Selected physical and chemical properties of individual groups of polybrominated biphenyls.

Properties	HexaBB	OctaBB	NonaBB	DecaBB
Molecular formula	$C_{12}H_4Br_6$	$C_{12}H_2Br_8$	$C_{12}H_1Br_9$	$C_{12}Br_{10}$
Relative molecular weight	627.4	785.2	864.1	943.0
Major congeners	153, 169	194	206, 207, 208	209
Melting point (°C)	124–128	200–250	220–290	380–386
Decomposition point (°C)	300–400	435	435	395–400
Saturated vapour pressure (mPa)	0.69×10^{-9}	-	-	$<0.60 \times 10^{-9}$
Solubility in water (mg/l)	11.10^{-3}	$30–40.10^{-3}$	unsoluble	$<30.10^{-3}$
log K_{OW}	7.20	-	-	8.58

similarity of PBBs and PCBs, PBB substances can act by the same mechanisms. The lethal dose LD_{50} in laboratory animals after oral administration of commercial mixtures indicates low acute toxicity of these substances (LD_{50} > 1 g/kg body weight). Experimental toxicological tests on laboratory animals have shown a range of adverse effects of prolonged use of PBB mixtures, such as weight loss, dehydration of the body, reproductive disorders, morphological and histopathological changes in the liver and kidneys, thymus shrinkage and swelling of the thyroid gland. PBBs also belong to a group of substances which interfere with the hormone system of the body. Some reports on the carcinogenicity of PBBs suggest that these compounds are not mutagenic themselves, but that their presence supports the carcinogenicity of other substances, such as nitrosamines and polycyclic aromatic hydrocarbons (PAHs).

The toxicities of individual PBB congeners may vary considerably; the most toxic substances are coplanar congeners, which lack a substituent in the *ortho*-position. Also, the degree of bromination can affect the toxicity. The most toxic substance is 3,3′,4,4′,5,5′-hexabromobiphenyl (BB 169), which is present in low concentrations in a commercial mixture under the trade name FireMaster. Conversely, the main component of this mixture, 2,2′,4,4′,5,5′-hexabromobiphenyl (BB 153), is relatively non-toxic.

Some PBBs are considered possible precursors of toxic polybrominated dibenzo-*p*-dioxins (**12-138**) and dibenzofurans (**12-139**), which are formed during combustion. The formation of degradation products of PBBs depends generally on the temperature, the amount of oxygen present and certain other factors. A study of the pyrolysis of the commercial mixture FireMaster BP-6 in the absence of oxygen at 600-900 °C showed the formation of bromobenzenes and lower brominated biphenyls, but polybrominated furans did not result. Pyrolysis in the presence of oxygen at 700–900 °C, however, yielded di- to heptabromodibenzofurans.

Mitigation

In 2006, EFSA recommended monitoring of the PBB congener 153, a marker of contamination by this group of POPs.

12.4.3.1.3 Tetrabromobisphenol A

Tetrabromobisphenol A (TBBPA, **12-134**) is currently the world's most widely used brominated flame retardant. It is produced industrially by bromination of bisphenol A, for example using 50% hydrobromic acid (HBr) in the presence of a solvent, usually carbon tetrachloride or its mixture with water. TBBPA is a colourless, crystalline or amorphous substance containing 58.7% w/w of bromine. A summary of selected physico-chemical properties of TBBPA is given in Table 12.51.

TBBPA has found use as a reactive flame retardant in the production of epoxy and polycarbonate resins added to circuit boards used in computers and in many other devices. In about 10% of cases TBBPA is used as an additive flame retardant, especially in the production of acrylonitrile–butadiene–styrene polymers, polystyrene, papers, textiles, televisions, office equipment and others.

TBBPA is not subject to the process of biomagnification and the main source of exposure is considered to be inhalation. It is assumed that it does not contribute to contamination of the environment as it is easily degraded by UV radiation and various bacteria. The main photodegradation product is 2,4,6-tribromophenol, which is accompanied by di- and tribromobisphenol A, dibromophenols, 2,6-dibromo-4-(bromoisopropylene)phenol, 2,6-dibromo-4-(dibromisopropylene)phenol and 2,6-dibromo-1,4-hydroxybenzene.

Acute toxicity of orally administrated TBBPA to laboratory animals is low (LD_{50} for rats is >5 g/kg body weight), with very little or no effects on body weight, organ abnormalities and behaviour. By contrast, absorption from water by fish is very fast and fish exposed to TBBPA (half-life of its degradation is up to several hours) often have jerking movements, seizures, darker colour, abnormal breathing and various inflammations, increased concentration of thyroid hormone thyroxine in plasma and, in some fish, reduced egg production and juvenile survival.

Table 12.51 Selected physical and chemical properties of tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD).

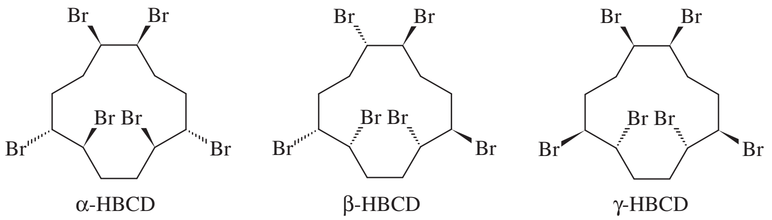
Properties	TBBPA	HBCD
Molecular formula	$C_{15}H_{12}Br_4O_2$	$C_{12}H_{18}Br_6$
Relative molecular weight	543.9	641.7
Melting point (°C)	181–182	185–200
Decomposition point (°C)	316	230
Saturated vapour pressure (mPa)	<0.1	0.63×10^{-7}
Solubility in water (mg/l)	4.16	0.0034
log K_{OW}	4.5	5.6

12.4.3.1.4 Hexabromocyclododecane

1,2,5,6,9,10-Hexabromocyclododecane (HBCD, **12-135**) is a compound with a bromine content of 74.7%. Although 16 different diastereoisomers are known, the commercially manufactured product is a mixture of only three isomers: α (optical antipodes α_1 and α_2), β (β_1 and β_2) and γ (γ_1 and γ_2) (**12-140**). A summary of certain physico-chemical properties of HBCD is shown in Table 12.51.

HBCD is produced industrially by bromination of cyclododecane, or by the addition of bromine to (1*E*,5*E*,9*Z*)-cyclododeca-1,5,9-triene. In both cases a mixture of 1,2,5,6,9,10-hexabromocyclododecane stereoisomers results.

Individual diastereoisomers differ in their melting point temperatures (α: 172 °C, β: 169 °C, γ: 209 °C) and their solubility in water (α: 48.8 μg/l, β: 14.7 μg/l, γ: 2.08 μg/l). At temperatures higher than 160 °C, β- and γ-isomers rearrange to the most thermodynamically stable α-isomer. Commercially used HBCD is produced in two forms, as low melting point and high melting point products, which differ from each other in the content of diastereoisomers. The low melting point mixture contains 70–80% γ-isomer and



12-140, hexabromocyclododecane diastereoisomers

20–30% α- and β-isomers, while the high melting point mixture may contain more than 90% γ-isomer.

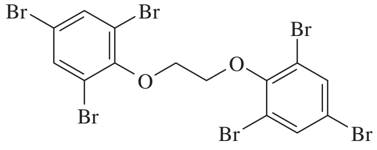
HBCD has been used as an additive flame retardant for more than 20 years. Its main use is in the construction industry, where it is used in polystyrene foams, which are a part of the thermal isolations, and even small concentrations provide sufficient protection against burning. The second major application of HBCD is in the upholstery and textile industries. Products in which the HBCD occurs, for example, include upholstered furniture, various textiles, car seats and upholstery, insulation in trucks and caravans as well as many types of building materials. Unlike other flame retardants, HBCD is not used in electronic circuits.

Currently there is very little knowledge about the possible risks from the use of HBCD. Tests on laboratory animals have found a very rapid absorption in the digestive tract and subsequently elevated levels of HBCD in the blood and certain organs. The acute toxicity of HBCD to mice is very low (LD_{50} for rats is >10 g/kg body weight by oral administration and >20 g/kg body weight for dermal application). At high doses, HBCD can damage the skin or eyes. Prolonged exposure in rats causes an increase in liver weight, reproductive disorders, and affects the nervous and hormonal systems.

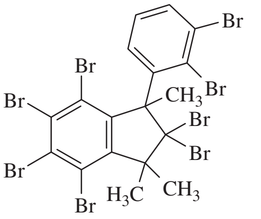
HBCD accumulates in soil, sediments and airborne dust, and is rapidly absorbed in the gastrointestinal tract and accumulates in the fatty tissue of organisms, but has a very short half-life. For example, in fish the half-life of HBCD is only one day. Representation of the individual isomers in the sediments reflects the compositions of commercial mixtures, while the tissues of aquatic animals, especially fish, have a quite different composition of isomers. The dominant substance is the α-isomer; the γ-isomer is present in much smaller amounts and the content of the β-isomer remains virtually constant, which can be explained by the almost selective biotransformation of the γ-isomer to the α-isomer.

12.4.3.1.5 Other flame retardants

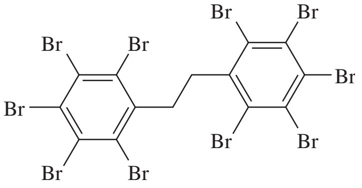
As a result of both gradual voluntary production cuts and legislative measures, production of technical mixtures of PBDEs has decreased and conversely there have been increases in the production of alternative non-PBDE flame retardants. The alternative retardants include bis(2,4,6-tribromophenoxy)ethane (BTBPE, **12-141**), octabromo-1-phenyl-1,3,3-trimethylindane (Br indane, **12-142**), decabromodiphenylethane (DBDPE, **12-143**) and certain other compounds. These substances have similar properties to PBDEs, contain an aromatic nucleus, more bromine atoms and are rarely soluble in water. Some of their physico-chemical properties are summarised in Table 12.52.



12-141, bis(2,4,6-tribromophenoxy)ethane



12-142, octabromo-1-phenyl-1,3,3-trimethylindane



12-143, decabromdiphenylethane

BTBPE is used as an additive flame retardant, replacing octaPBDE, for polystyrene and acrylonitrile–butadiene–styrene products. BTBPE was found in the eggs of birds and especially in the dust and air in plants for the liquidation of electronic waste. Animal studies indicate minimum BTBPE absorption in the digestive tract. The major part of BTBPE is probably excreted, partly as metabolites, such as hydroxylated BTBPE and 2,4,6-tribromophenol.

Br indane is an additive flame retardants, which is manufactured under the name of FR-1808 by reaction of 1-phenyl-1,3,3-trimethylindane with bromine through catalysis with Lewis acids. It is used in technical resins and polystyrene foams. Br indane is kinder to the environment than PBDEs.

DBDPE is an additive flame retardant with similar uses as decaBDE. It is employed in acrylonitrile–butadiene–styrene products, and is added to polystyrene and insulation materials. DBDPE has been detected in air, soil and sludge at concentrations one order of magnitude higher than BTBPE. DBDPE enters the body in small quantities due to its higher molecular weight.

Table 12.52 Selected physical and chemical properties of new brominated flame retardators.

Compound	Molecular formula	M_r	log K_{ow}	Saturated vapour pressure (Pa)
Br indane	$C_{18}H_{12}Br_8$	867.5	-	-
BTBPE	$C_{14}H_8Br_6O_2$	687.6	9.15	30.99
TBB	$C_{15}H_{18}Br_4O_2$	549.9	8.75	-
DBDPE	$C_{14}H_4Br_{10}$	971.2	7–10	0.000 001
TBPH	$C_{24}H_{34}Br_4O_4$	706.1	11.95	0

12.4.3.2 Perfluoroalkyl and polyfluoroalkyl substances

Per(poly)fluoroalkyl substances (PFASs) are the result of anthropogenic activities in the latter half of the 20th century. PFASs include many different groups of compounds starting from one-carbon compounds and ending with polymeric substances. Attention was initially focused on research on volatile chlorofluorocarbons (CFCs, known as **freons** by DuPont's brand name), which are also known as greenhouse gases. Polytetrafluoroethylene (PTFE) is a synthetic polymer of tetrafluoroethylene with numerous applications. The best known brand name of PTFE is **Teflon** from the DuPont Company.

The term per(poly)fluoroalkyl substances (PFASs) is the common name for a group of synthetic fluorinated chemicals, including oligomers and polymers. The major areas of use for currently available PFASs are in fire-fighting foams and the chromium-plating industry. PFASs are persistent compounds with bioaccumulation potential that have a negative impact on the environment. Their presence has been confirmed in abiotic and biotic components of the environment around the world.

12.4.3.2.1 Structure and nomenclature

From a chemical point of view, PFASs comprise carbon chains of varied length, in which hydrogen atoms have been wholly (perfluorinated) or partly (polyfluorinated) replaced by fluorine atoms. PFASs include several hundred compounds divided into 23 categories. Important subgroups are per(poly)fluorinated organic surfactants and organic polymers. Commercial products contain per(poly)fluorinated alkyl chains of varying lengths with 4 to 20 carbon atoms. Most of the available data, however, relates to compounds having eight carbon atoms. An overview of these substances is given in Table 12.53. The structure of these groups of compounds is represented by formulae **12-144**. In the environment PFASs are degraded almost exclusively to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which are, therefore, the most significant fluorinated contaminants.

12.4.3.2.2 Production

Two main ways of fluorination are used in the industrial production of PFASs. Branched and unbranched fully fluorinated products containing a sulfonic group are synthesised electrochemically; so-called telomerisation (polymerisation in the presence of a chain transfer agent) forms products that are not fully fluorinated, but have a linear perfluorinated alkyl chain with an ethylene group and another functional group. The most important difference between these two production processes is the quality of the final product. Electrochemical fluorination may be used to produce all types of fluorinated compounds, depending on the starting material and its purity. This method was used, for example, by 3M. The telomerisation process is currently used by DuPont, Atofina, Clariant, Daikan and Asahi Glass. The products produced electrochemically contain up to 30% undesirable branched compounds. Substances produced by telomerisation contain very few byproducts, but those which are produced are easy to separate from the desired product.

12.4.3.2.3 Properties

Physical and chemical properties

Perfluorinated compounds have special physico-chemical properties, which make them valuable for use in various industries. They are chemically inert, highly thermally stable and reduce surface tension. They repel both water and oil, so they are both hydrophobic and oleophobic substances. Table 12.54 summarises some physico-chemical properties of perfluorooctane sulfonate (PFOS) potassium salt and perfluorooctanoic acid (PFOA).

In the past PFASs have found use as:

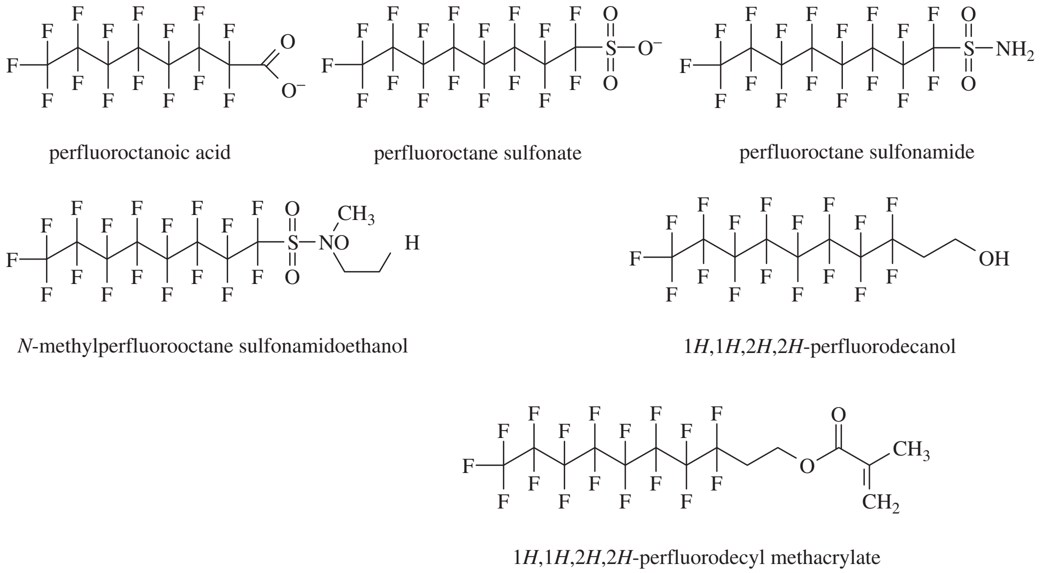
- surface treatment of carpets, fabrics, leather, paper and packaging materials
- manufacture of paints and additives for coatings
- manufacture of detergents for domestic and industrial use
- manufacture of pesticides.

Perfluorinated surfactants do not behave like common organic pollutants. They do not tend to accumulate in adipose tissue or to adsorb, for example to deposits. However, electrostatic interactions play an important role in their distribution in the environment. After entering the organism, these substances preferentially accumulate in the blood plasma and liver (apparently

Table 12.53 Overview of electrochemically produced per(poly)fluoroalkyl substances.

Name	Abbreviation	Classification[a]
Perfluoroctanoic acid	PFOA	1, 3
Perfluorobutane sulfonate	PFBS	1
Perfluorohexane sulfonate	PFHxS	1
Perfluorooctane sulfonate	PFOS	1
Perfluorodecane sulfonate	PFDS	5
Perfluoroctane sulfonamide	PFOSA	5
Perfluorooctane sulfonyl fluoride	PFOSF	2
N-Methylperfluoroctane sulfonamide	*N*-MeFOSA	2
N-Ethylperfluoroctane sulfonamide	*N*-EtFOSA	2
N-Methylperfluoroctane sulfonamidoethanol	*N*-MeFOSE	2
N-Ethylperfluoroctane sulfonamidoethanol	*N*-EtFOSE	2
1*H*,1*H*,2*H*,2*H*-Perfluoroctanol	6:2 FTOH	2
1*H*,1*H*,2*H*,2*H*-Perfluorodecanol	8:2 FTOH	2
1*H*,1*H*,2*H*,2*H*-Perfluorododecanol	10:2 FTOH	2
1*H*,1*H*,2*H*,2*H*-Perfluoroctyl acrylate	6:2 FTA	4
1*H*,1*H*,2*H*,2*H*-Perfluordecyl acrylate	8:2 FTA	4
1*H*,1*H*,2*H*,2*H*-Perfluoroctyl methacrylate	6:2 FTMA	4
1*H*,1*H*,2*H*,2*H*-Perfluorodecyl methacrylate	8:2 FTMA	4

[a] Criterion for classification: 1 = probable degradation product, 2 = important intermediate in the manufacture, 3 = important commercial product, 4 = important monomer for the production of polymers, 5 = data not available.



12-144, structures of per(poly)fluorinated substances

Table 12.54 Selected physical and chemical properties of perfluorooctane sulfonate (PFOS) potassium salt and perfluorooctanoic acid (PFOA).

Properties	Values	
	PFOS	PFOA
Molecular formula	$C_8KSF_{17}O_3$	$C_8HF_{15}O_2$
Relative molecular weight	538.21	414.07
Melting point (°C)	>400	45–50
Boiling point (°C)	Not quantifiable	189–192
Saturated vapour pressure (mPa)	0.000 044 (21°C)	1.33 (25 °C)
Solubility in water (mg/l)	680 (25 °C)	3400
log K_{ow}	4.13	Not quantifiable

through interactions with phospholipids). The bioconcentration factor of perfluorooctane sulfonate (PFOS) is significantly higher than that of perfluorooctanoic acid (PFOA). For example, the bioconcentration factor of PFOS was 4300 in the blood of rainbow trout, while that of PFOA was 27. A considerable amount of PFOS is also deposited through the application of sewage sludge on agricultural land.

12.4.3.2.4 Occurrence and main sources

Release into the environment

Per(poly)fluorinated substances are emitted directly to the environment at the place of manufacture (mostly sorbed on dust particles) and during normal use of products containing PFASs, both in industry and in households. PFOS and related substances may also be emitted from wastewater treatment plants and landfills, where elevated concentrations have been observed. Increased concentrations of PFOS were found mainly in waste water and water leaking from landfills. Fire foams are also a significant source of PFOS emissions. After penetration into the environment, per(poly)fluorinated substances are adsorbed to the organic fraction of the sediment or bioaccumulate in living organisms. A major obstacle in estimating the penetration of PFOS into the environment is degradation of PFOS to several related perfluorinated compounds. The extent of the degradation reactions is not yet fully understood. For example, higher concentrations of PFOS were found in water coming from sewage treatment plants than in the water going into the plants, which points to a possible conversion of perfluorinated substances present in the water to PFOS.

Biodegradation

Owing to the high energy of the C–F bond, many fluorinated organic compounds, except acrylates, are resistant to hydrolysis, photolysis and biodegradation. Figure 12.53 is an example of microbial degradation of *N*-ethylperfluorooctane sulfonamidoethanol (*N*-EtFOSE) in wastewater, which can document biotransformation of some perfluorinated compounds. The end products are PFOS and PFOA, which are stable under aerobic and anaerobic conditions and are not further degraded. Biodegradation of *N*-methylperfluorooctane sulfonamidoethanol (*N*-MeFOSE) also proceeds in the same way.

Contamination of animals

PFOS and related perfluorinated compounds have been found in many organisms around the world. Generally, they are found in the highest amounts in the tissues of predators at the top of the food chain, which have fish as the major food component. In addition to PFOS, perfluorooctane sulfonamide (PFOSA) is usually present, one of the precursors of PFOS. In fish there are often higher PFOSA concentrations than PFOS concentrations, whereas in mammals the opposite is true. This may indicate that mammals have metabolic apparatus that can metabolise PFOSA to PFOS.

In 2002 results were published obtained from the examination of marine mammals, birds, fish, snakes and amphibians (liver, egg yolks, muscle and blood plasma) from all over the globe, including areas of the North and South Poles. PFOS was found in 77% of samples of marine mammals, in 60% of samples of birds, in 38% of fish samples and in 100% of samples of minks and otters. The concentration of PFOS in the liver of seals from the Baltic Sea was 240 μg/kg, in the liver of Canadian polar bears 3100 μg/kg and 1200 μg/kg in the liver of North American minks. Samples from industrial areas, however, contained several times higher amounts of PFOS compared with samples from the Polar Regions. Table 12.55 presents the concentrations of PFOS and PFOA in fish, crustaceans and molluscs.

Human exposure

The first mention of the presence of organic fluorine compounds in human plasma dates back to 1968. Only in the mid-1990s, however, was the occurrence of perfluorinated compounds in plasma confirmed by identification of perfluorooctanoic acid (PFOA). Human exposure to PFASs, including PFOA and PFOS, probably occurs via different exposure pathways, including orally, dermally or by inhalation. Indirect factors such as place of residence, age, type of PFAS and others may also affect the extent of exposure. In general, the concentrations of PFOS in human plasma are higher than concentrations of PFOA. Lower concentrations are found in Europe and Asia, with higher concentrations in the United States and Australia. The exceptions are the Nordic countries Denmark and Sweden, where the findings are comparable to those in the United States. Amounts of PFASs in plasma of men in Germany were twice as high as in the plasma of women and children. In occupationally exposed people, higher amounts of PFASs are generally found (up to thousands of μg/l) with no evidence of association between age and sex, which may be related to the fact that these compounds have a tendency to bind to lipoproteins rather than to neutral lipids, as is the case with PCBs. Table 12.56 presents the concentrations of selected PFASs in breast milk, as an example.

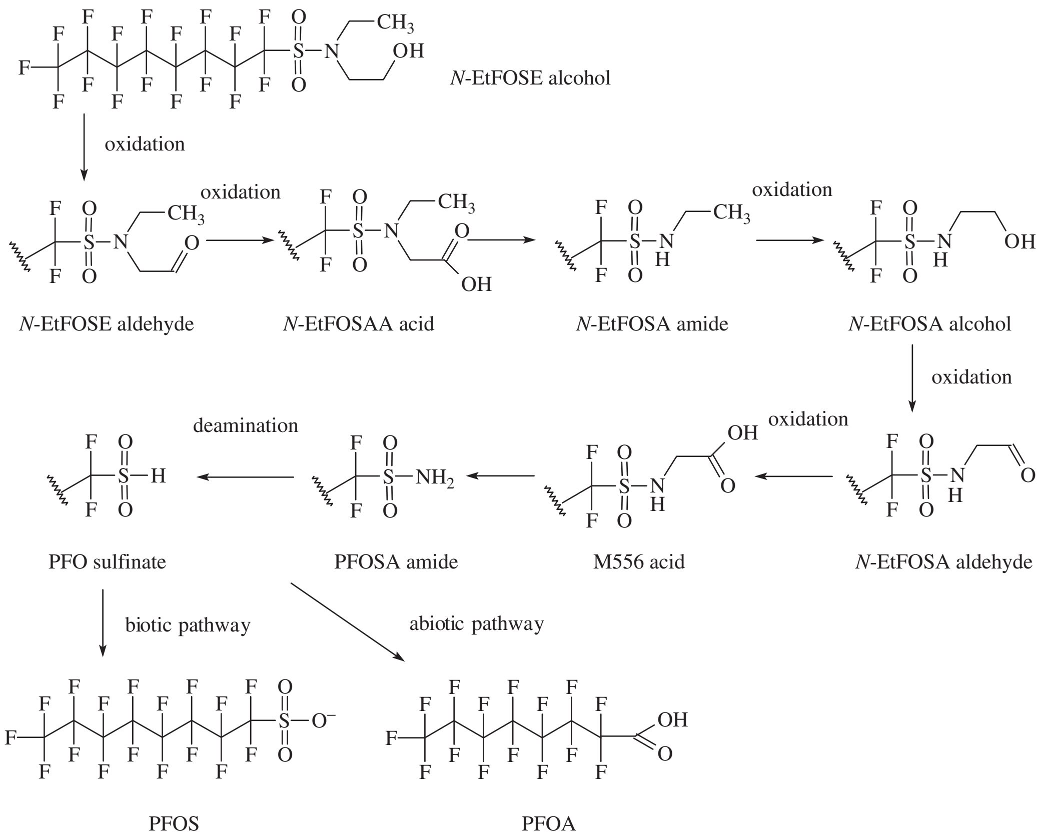


Figure 12.53 Microbial degradation of *N*-ethylperfluorooctane sulfonamidoethanol in sewage sludge.

Table 12.55 Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in aquatic fauna.

Continent	Species	PFOS (μg/kg)	PFOA (μg/kg)
Europe	Fishes	15.3	0.73
	Crustaceans	184	-
	Molluscs	69.1	-
Asia	Fishes	7.01	3.05
	Crustaceans	2.99	1.4
	Molluscs	5.44	5.2
North America	Fishes	129	1.56
	Crustaceans	0.35	0.17
	Molluscs	0.28	-

Two recent studies from the United Kingdom and Spain on the occurrence of PFASs in the food basket proved that the most important sources of human exposure are fish, fish products and drinking water (surface water is considered one of the major sources of perfluorinated substances). The PFASs occurring in the highest concentrations were PFOS and PFOA, which are the substances most commonly found in the environment. Table 12.57 summarises the amounts of PFOS and PFOA in selected foods in Spain, as an example.

12.4.3.2.5 Health and toxicological evaluation

The mechanisms of the toxic effects of perfluorinated compounds are not yet fully understood. Among the possible harmful effects may be effects on the transport and metabolism of fatty acids in the biological membranes or the bioenergetic processes in the mitochondria. PFOS is practically non-toxic (also in evaluation of subchronic and chronic toxicities) for freshwater algae and higher plants, but shows a slight toxicity to invertebrates. Fishes are much more sensitive to the presence of this substance than algae, plants and invertebrates (the lowest LC_{50} value was 7.8 mg/l for rainbow trout). PFOA is also virtually non-toxic for all tested species of freshwater organisms (bacteria, algae and fish).

After PFOS enters the human body orally, it is relatively easily absorbed and distributed mainly to the liver and blood serum, but is not metabolised. It is excreted in the urine and faeces, but its excretion is very slow. It is estimated that half of the received dose may be removed from the human body in approximately 9 years. PFOA can enter the human body not only orally, but also (to a lesser extent) by inhalation and dermal absorption. It is not accumulated in the adipose tissue, but is covalently bound to macromolecules, especially proteins in the liver, plasma and testes of males. It is not metabolised and is excreted from the body in urine and faeces. It is estimated that half of the ingested amount can be removed from the human body in 1–3 years.

Table 12.56 Concentration of selected per(poly)fluorinatedalkyl substances (PFASs)[a] in breast milk (μg/l).

Country	PFOS	PFOA	PFHxS	PFOSA	PFBS
China	0.045	0.110	0.009	-	0.003
Germany	0.116	0.077	-	-	-
Hungary	0.317	0.077	-	-	-
Japan	0.232	0.078	0.008	-	<0.001
Malaysia	0.121	<0.043–0.090	0.006	-	<0.001–0.017
Philippines	0.098	<0.043–0.183	0.016	-	<0.001–0.017
Sweden	0.201	-	0.085	0.013	-
USA	0.131	0.044	0.015	-	<0.01–0.020
North Carolina (USA)	21.900	3.990	1.940	0.070	-

[a] See Table 12.53.

Table 12.57 Amounts of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in Spainish foods.

Foods	PFOS (μg/kg)	PFOA (μg/kg)	Foods	PFOS (μg/kg)	PFOA (μg/kg)
Fruits	<0.017	<0.036	Sea fish	0.407	<0.065
Vegetables	0.022	<0.027	Canned fish	0.271	<0.126
Legumes	<0.027	<0.045	Eggs	0.082	<0.055
Cereal products	<0.069	<0.080	Milk, full fat	<0.014	0.056
Pork meat	0.045	<0.053	Milk, reduced fat	<0.019	<0.028
Veal meat	0.028	<0.034	Dairy products	0.121	<0.040
Chicken	0.021	<0.067	Margarines	<0.034	<0.115
Seafood	0.148	<0.029	Vegetable oils	<0.099	<0.247

12.4.3.2.6 Mitigation

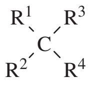
Contamination of the environment and the human food chain with PFASs influenced the chemical industry to such an extent that the world's largest producer of PFASs, the sole US manufacturer 3M, announced a voluntary shutdown of production in 2000. The U.S. Environmental Protection Agency (EPA) issued two Significant New Use Rules (SNURs) under the Toxic Substance Control Act (TSCA) in 2002 to restrict 88 PFOSs-related chemicals. SNURs allow only three specific, technically essential low volume, low exposure, low release uses to continue: for the photographic/imaging industry, the semiconductor industry and the aviation industry. The final SNUR for 183 PFAS chemicals was published in 2007. The goals were to achieve, no later than 2010, 95% reduction in emissions to all media and products the contents of PFOA, PFOA precursor chemicals and related higher homologue chemicals measured from a year 2000 baseline, and to work toward elimination of PFOA and PFOA precursors and related higher homologue chemicals from emissions and products by 2015.

The European Food Safety Authority (EFSA) has been involved in PFASs in food since 2004, when recommendations to EU member states to monitor these substances were first issued. In 2009, PFOS, its salts and perfluorooctane sulfonyl fluoride (PFOSF) were subjected to restrictions on production and use and were added to Annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs). For PFOS, the lowest No-Observed-Adverse-Effect Level (NOAEL) was established at 0.03 mg/kg body weight per day and a TDI was derived at 150 ng/kg body weight. Based on the occurrence of PFOS in fish and fishery products and drinking water, together with consumption data from four Member States, an indicative figure of 60 ng/kg body weight per day for human exposure was selected. The estimated indicative exposure of high consumers of fish and fishery products is approximately three times as high (200 ng/g body weight per day). Non-food sources of PFOS were estimated to contribute in the order of 2% or less to average

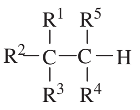
dietary exposure. Drinking water appears to contribute less than 0.5%. For PFOA, an indicative figure of 2 ng/kg body weight per day was established for average human exposure (6 ng/g body weight per day for the exposure of high consumers of fish and fishery products). At these estimated intakes, non-food sources could contribute up to 50% of the average dietary exposure, whereas drinking water would contribute less than by 16%. The TDI value was estimated at 1.5 μg/kg body weight, and the average daily intake for the European population was estimated to be in the range of 2–6 ng/kg body weight.

12.5 Chlorinated aliphatic hydrocarbons

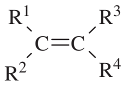
Chlorinated Aliphatic Hydrocarbons (CAHs), a family of compounds that are commonly used in many areas of human activity, are increasingly being detected in soil and groundwater. The most prevalent of these CAHs are tetrachloroethylene (perchloroethylene, PCE), 1,1,2-trichloroethylene (TCE) and 1,1,1-trichloroethane (TCA) illustrated in formulae **12-145** to **12-147**. At the beginning in the 1960s, CAHs were used as industrial solvents primarily for degreasing in the dry cleaning, electronics, industrial manufacturing and machine maintenance industries, and as solvents for varnishes and paints, fats, waxes, resins and alkaloids. For example, dichloromethane (methylene chloride, DCM) is used in the manufacture of urethane polymers and aerosols, and to extract caffeine during the manufacture of decaffeinated coffee. Trichloroethylene is used as a heat transfer medium, as a raw material for the production of trichloroacetic acid and fluorinated hydrocarbons, and together with tetrachloroethylene is used as an industrial solvent for fats, oils, tars, rubber and gums and as a cleaning and degreasing agent in the metals-processing industry. Trichloromethane (chloroform) and 1,1,2-trichlorethylene were previously used as inhalation narcotics, while carbon tetrachloride



12-145, chlorinated methane derivatives
dichloromethane, $R^1 = R^2 = Cl$, $R^3 = R^4 = H$
trichloromethane, $R^1 = R^2 = R^3 = Cl$, $R^4 = H$
tetrachloromethane, $R^1 = R^2 = R^3 = R^4 = Cl$



12-146, chlorinated ethane derivatives
1,1-dichloroethane, $R^1 = R^2 = Cl$, $R^3 = R^4 = R^5 = H$
1,2-dichloroethane, $R^1 = R^4 = Cl$, $R^2 = R^3 = R^5 = H$
1,1,1,-trichloroethane, $R^1 = R^2 = R^3 = Cl$, $R^4 = R^5 = H$
1,1,2-trichloroethane, $R^1 = R^2 = R^4 = Cl$, $R^3 = R^5 = H$
1,1,2,2-tetrachloroethane, $R^1 = R^2 = R^4 = R^5 = Cl$, $R^3 = H$



12-147, chlorinated ethane (ethylene) derivatives
trichloroethylene, $R^1 = R^2 = R^3 = Cl$, $R^4 = H$
tetrachloroethylene, $R^1 = R^2 = R^3 = R^4 = Cl$

and 1,1,2-trichlorethylene were used as anthelmintic agents (against foodborne diseases called helmintoses caused by parasitic worms), especially in veterinary medicine. Later they were forced out by the less toxic perchloroethylene. As a result of leakage of CAHs from storage tanks and machinery, they are now appearing in groundwater and also in foods at concentrations which may be unhealthy and even hepatotoxic, nephrotoxic or carcinogenic. Some of their properties are shown in Table 12.58.

Food contamination can occur in various ways. In the vicinity of factories for dry cleaning of clothing and textiles high concentrations of trichlorethylene (together with trichloromethane) in butter and lard (up to 0.76 mg/kg) were found. Other solvents found in foods include chloroethylene (vinyl chloride), which is used to make polyvinyl chloride (PVC), 1,1-dichloroethylene (vinylidene chloride), used to produce copolymers with vinyl chloride (see Section 12.9.1.5), and (*Z*)- and (*E*)-1,2-dichloroethylene, which are used for various syntheses and as solvents.

A number of chlorinated hydrocarbons arise during reactions of alkanes and alkenes with various chlorinated compounds, or by degradation of other chlorinated compounds. For example, as components of acid protein hydrolysates by traditional technology (hydrolysis with hydrochloric acid), 1,2-dichloropropane and 1,3-dichloropropane (see Section 12.2.4.1) are produced, the precursors of which are residual triacylglycerols found in oilseed meals.

Most CAHs damage the kidneys, cause liver parenchyma and have carcinogenic effects. The legislation defines the permissible amounts of chlorinated aliphatic hydrocarbons in food generally as the sum of all the substances listed in Table 12.58. Minimum amounts are: for milk 0.001 mg/kg, dairy products and fish (0.002 mg/kg), cheeses and poultry (0.003 mg/kg), potatoes (0.004 mg/kg), spirits (0.005 mg/kg), eggs, flour, butter, lard and fats (0.01 mg/kg). The highest levels are set for decaffeinated coffee (3 mg/kg).

12.6 Pesticides

Efforts to protect food supply against attacks by various pests have been documented since ancient times. The Chinese treated cereals and other plants seeds with soda ash and olive oil before storing and used preparations containing arsenic to protect crops during the growing season. The Ancient Greeks and Romans used the antiseptic properties of combustion gases resulting from the burning of sulfur to ward off pests. Substances acting against insect pests are found in many plants that have been used in the past in many countries. Traditional natural pesticides include the alkaloids nicotine and anabasine (see **10-7**), so called **nicotinoids**,

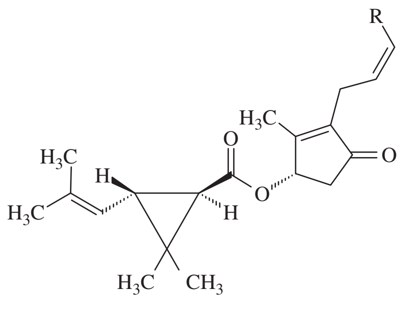
Table 12.58 Overview of physical and chemical and other properties of chlorinated aliphatic hydrocarbons.

Compound	M_r (Da)	Melting point (°C)[a]	Boiling point (°C)[a]	Solubility[b]	LD_{50} (ml/kg)[c]
Dichloromethane	84.9	−95	39.8	50	1.6
Trichloromethane	119.4	−63.5	61.5	200	2.2
Tetrachloromethane	153.8	−23	76.7	2000	2.9
1,1-Dichloroethane	99.0	−98	57.3	200	-
1,2-Dichloroethane	99.0	−40	83.5	120	0.8
1,1,1-Trichloroethane	133.4	−32.5	74.1	700	0.2
1,1,2-Trichloroethane	133.4	−35	113.5	700	0.6
1,1,2,2-Tetrachloroethane	167.9	−44	146.5	350	0.2
Trichloroethylene	131.4	−85	86.9	1000	4.9
Tetrachloroethylene	165.8	−22	121.0	10 000	8.9

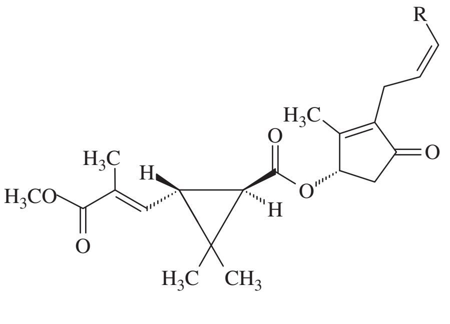
[a] At normal pressure.
[b] In ml of water in which dissolves 1 ml of solvent at 25 °C.
[c] For rats (orally).

contained in extracts from leaves and roots of tobacco, mainly from arborescent tobacco species *Nicotiana glauca* (Solanaceae) native to Argentina. Anabasine also occurs in the leaves of *Anabasis aphylla*, a plant belonging to the Amaranthaceae (formerly Chenopodiaceae) family, which grows in the steppes of Central Asia. Plants of the genus *Pyrethrum* (Asteraceae), in particular *P. cinerariaefolium* originating from the Balkans and the Adriatic Sea islands and *P. carneum* growing in the Caucasus and around the Black Sea, contain active components called **pyrethroids** (**12-148**), which have a strong insecticidal effect. Extracts (called pyrethrum) are a mixture of three structurally related esters of a monoterpenoid called chrysanthemic acid (pyrethrins I) and three esters of pyrethrinic acid (pyrethrins II). Pyrethrins later became prototypes of synthetic pyrethroids. One of the most significant natural insecticides and acaricides (pesticides that kills mites and ticks) is (5′*R*,6a*S*,12a*S*)-rotenone (**12-149**) from the group of isoflavones (see **10-95**) called **rotenoids** that are highly toxic to all life forms. Rotenoids occur in some plants of the family Fabaceae, for example in the roots of *Derris elliptica* lianas native to Indonesia and liana species *Lonchocarpus utilis*, *L. urucu* and *L. nicou*, which are native to South America. Powders of lianas are used by natives as a fish poison, but its toxic effects on insects and mites have also been shown. The extract of quassia (also known as bitterwood, *Quassia amara*, Simaroubaceae) likewise has an insecticidal effect. It grows in Central and South America. This extract contains a group of bitter substances called **quassinoids**. A typical representative is quassin (**8-249**).

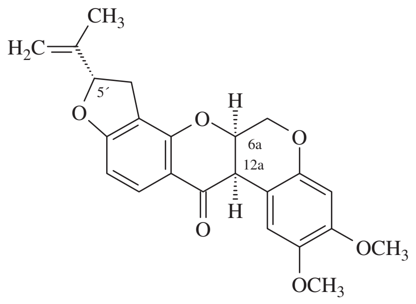
The beginning of a scientifically conceived, systematic study of the applications of chemical compounds for crop protection dates back to the mid-19th century. In 1867, the so-called Paris green began to be used as an insecticide and rodenticide (Paris Green is also known as Emerald Green, Schweinfurt Green, Imperial Green, Vienna Green, Mitis Green and Veronese green), which is copper(II) acetoarsenite, $4Cu^{2+}(As_3O_6^{3-})_2(CH_3COO^-)_2$. For the prevention of fungal infections in vineyards and potatoes, the Bordeaux mixture (also called Bordo Mix) is used, which is a mixture of copper



12-148, pyrethrins I
pyrethrin I, R = $CH{=}CH_2$
jasmolin I, R = CH_2CH_3
cinerin I, R = CH_3



pyrethrins II
pyrethrin II, R = $CH{=}CH_2$
jasmolin II, R = CH_2CH_3
cinerin II, R = CH_3



12-149, rotenone

sulfate, lime and water invented in the Bordeaux region of France in 1886. This fungicide has been used for over a century and is still used today. A number of other inorganic compounds have since been tested; however, a significant milestone was the introduction of organic mercury compounds for the protection of seeds in 1913. A significant expansion of the use of organic compounds that had pesticidal effects began in the 1930s. Among the first herbicides designed to kill weeds in fields were dinitroderivatives of *o*-cresols, the fungicide thiram from the dithiocarbamates group and pentachlorophenol to combat wood-destroying fungi. One of the first insecticides was the organophosphate tetraethyl pyrophosphate (TEPP).

In 1939, the highly efficient contact insecticide DDT (an abbreviation of the technically incorrect name dichlorodiphenyltrichloroethane) was introduced to the market. Its use, as well as the use of several other organochlorine compounds, spread worldwide in the following years. Important active substances that appeared on the market in the period after World War II include carbamate insecticides and herbicides. Also significant was the discovery of herbicidal phenoxyacetic acids, which represent the first group of the so-called hormonally active pesticides. Around the middle of the last century a number of other biologically active substances were discovered, many of which, such as herbicides based on substituted urea, *s*-triazines (1,3,5-triazines), quaternary ammonium salts or insecticidal synthetic pyrethroids and many others, are still used in many countries around the world.

Alarming signals regarding the occurrence of residues of DDT and of other lipophilic organochlorine pesticides (such as aldrin and dieldrin) in virtually all parts of the global ecosystem and their adverse ecotoxicological effects that began to emerge in the 1960s, however, substantially contributed to the documentation of the potential risks arising from the use of pesticides. DDT and related compounds were progressively banned in almost all countries of the world. This helped initiate research aimed at studying the possibility of environmental protection, including human food chains, against continuing contamination by such persistent pollutants.

In general, the use of pesticides and related biologically active compounds has become an indispensable means of intensifying agricultural production. Besides agriculture, pesticides are also used in forestry and water management and in communal hygiene. The use of these agrochemicals not only provides an increased harvest, but very often manifests into higher nutritional, sensory and technological quality of treated crops. The FAO estimates that crop losses caused by various harmful agents worldwide, if protective agents are not used, could be as high as 30%, and in developing countries even higher. Of course, the extent and type of damage in the given region is subject to weather conditions and the character of the agroecosystem.

In addition to the indisputable positive aspects arising from the use of pesticides, however, there are also negative impacts of the chemical processing of agriculture on the biotic components of the environment, including humans. According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 most persistent organic pollutants are pesticides. Consumers are particularly sensitive to the potential presence of pesticide residues in food, which is one of the main incentives for the increasing interest in foodstuffs produced from so-called ecological and organic farming, which exclude the use of synthetic pesticides.

Knowledge of the basic physico-chemical properties of pesticides, methods of their application, their fate in the environment and especially in food chains, along with information on their changes during storage and processing of food materials and products, is an essential prerequisite for successful adherence to legislative measures and the related reduction of the risk of exposure of consumers to possible pesticide residues.

12.6.1 Classification and nomenclature

A pesticide is any substance or mixture of substances specifically intended to prevent, repel, destroy or lessen the effect of pests, undesirable microorganisms, plants and animals during the production, storage, transport, distribution and processing of agricultural commodities, foods and feeds. The term also includes compounds administered to animals for the control of ectoparasites, growth regulators, desiccants and germination inhibitors applied to crops either before or after harvest.

Pesticides are substances representing a wide range of chemical compounds, often of very complex structure. To facilitate communication, pesticides are known by their **common name**, **trade name** and by their **chemical name**. The common name is name of the active ingredient, the biologically active substance that controls the pest. The trade name is the prominent brand name that the manufacturer gives to the pesticide. Pesticides with different trade names can contain the same active ingredient or ingredients. In practice, the names predominantly used are the common and the trade names. The chemical name is the name of the chemical structure of the active ingredient and is used by scientists. Common names of pesticides and other agrochemicals have been adopted by the Technical Committee of the International Organization for Standardization, created in 1953 (ISO/TC 81). Principles for the selection of common names of pesticides and other agrochemicals are explained in ISO 257:1988, and revised by ISO 257:2004. For example, fungicide containing captan as the active ingredient, the chemical name of which is *N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide, is called by the trade name Captan or Maestro. The insecticide carbaryl (1-naphthyl methylcarbamate) has the trade names Sevin, Vet-Tek amongst others.

Table 12.59 Classification of pesticides by target harmful organisms.

Group of pesticides	Target harmful organism	Group of pesticides	Target harmful organism
Acaricides (miticides)	Mites	Ovicides	Insect eggs
Algicides	Algae	Larvicides	Insect larvae
Avicides	Birds	Adulticides	Adult insects (imago)
Bactericides	Bacteria	Molluscicides	Molluscs
Fungicides	Fungi, parasitic fungi	Nematicides	Worms
Herbicides	Weeds	Rodenticides	Rodents
Insecticides	Insect	Virucides	Viruses

At present, about 1000 compounds are registered globally that are used as pesticides or other agrochemicals (particularly as herbicides to protect cultivated plants). A common criterion used for their classification is the target (usually harmful) organisms. The most common types of pesticides used are listed in Table 12.59. Table 12.60 summarises the most important groups of pesticides used over the past decades, of which many are still in use. They are classified according to their biological effects. Naturally, the list does not include all registered pesticides, but focuses mainly on the new generation pesticides often referred to as **modern pesticides**. For completeness, substances that can generally be described as **traditional pesticides** or **historical pesticides** are listed. Their use globally is practically nil.

12.6.2 Properties and structures

12.6.2.1 Physical and chemical properties

Pesticides that have been applied in practice represent an extremely large and varied range of chemical compounds (Table 12.60), which is, of course, reflected in a wide variability in their physico-chemical properties. Detailed characterisation of individual compounds obviously goes beyond the scope of this book; therefore, comments here are restricted to some of the major groups of pesticides with regard to their fate in food chains and the changes taking place during technological or culinary processing of contaminated materials. The following characteristics are generally critical for the description and assessment of a pesticide's properties and its behaviour in different systems, and also to predict its levels in human nutrition.

Solubility in water is one of the key characteristics of pesticides that determines their distribution and the stability of the individual components in the environment and in plants. Retention (sorption) of particularly soluble polar pesticides to soil particles is relatively small, but they can penetrate into underground water resources. Polar pesticides are not very stable in the environment and are biodegradable; they can be easily hydrolysed and oxidised. Their limited stability is then the cause of a relatively rapid decrease of their residual amounts in contaminated agricultural products during thermal processing. On the other hand, the loss of polar pesticides by volatilisation from the environment is not significant (a certain prediction of their behaviour provides Henry's law constant, k_H). Pesticides with good solubility in water can generally be easily washed away from the surface of plants, but for lipophilic compounds this occurs with difficulty or not at all.

The saturated vapour pressure is another factor that is directly related to the fate of pesticides after their application. It is assumed that substances with saturated vapour pressure values lower than approximately 1×10^{-7} mPa are associated with solid particles; otherwise they are in the form of vapour. During culinary and technological processes such as drying, milling, baking or frying, higher losses of more volatile compounds can be expected. The extent of these losses, however, depends on the type of food.

The octanal–water partition coefficient K_{OW} in equilibrium at a given temperature usually correlates directly with the degree of pesticide sorption to soil particles and sediments. Generally, substances with a high K_{OW} value (e.g. classical organochlorine pesticides) have a high affinity for the lipidic components of living organisms and are accumulated. Particularly fat-soluble compounds typically have the pK_{OW} value (–log K_{OW}) higher than 4.

The dissociation constant K_a indicates the ability of pesticide residues to dissociate under normal environmental conditions (pH values are in the range of 5–8). In general, the degree of ionisation determines the solubilisation processes, evaporation from aqueous media and the extent of photolysis. Dissociation is obviously influenced by the sorption of pesticides to sediments and soil particles, and by their bioaccumulation.

The octanal–water partition coefficient K_{OC} normalised to the organic carbon content indicates the sorption ability of pesticides to soil or sediment particles. Pesticides with high values of K_{OC} are often very persistent in the environment, since the bound (adsorbed or absorbed) residue is immobilised and can only leach, evaporate or be biodegraded with difficulty.

The bioconcentration factor (BCF) of hydrophobic pesticides indicates the degree of their transition from the aqueous environment, and is a measure of their bioconcentration in the respective organism. The BCF value corresponds to the ratio of the equilibrium concentrations organism/water and is directly proportional to the values of pK_{OW}.

Table 12.60 Classification of pesticides into groups according to the target organism, application method, activity and structure.

Pesticides	Names of pesticides representing a given group[a]
Non-systemic insecticides	
Organochlorine compounds	Aldrin, DDT, dieldrin, dicofol, endosulfan, endrin, heptachlor, chlordan, γ-HCH (lindane), methoxychlor, toxaphen
Organophosphorus compounds	Azinphos-methyl, diazinone, dichlorvos, ethione, etrimphos, fenitrothione, fomophos, phosalone, chlorfenvinphos, chlorpyriphos, chlorpyriphos-methyl, quinalphos, malathione, mecarbam, methidathione, mevinphos, parathione-ethyl, parathione-methyl, pirimiphos-methyl, sulfotep, terbuphos, tetrachlorvinphos, tolclophos-methyl, triazophos
Carbamates	Phenoxycarb, formethanate, carbaryl, methiocarb, methomyl, propoxur
Synthetic pyrethroids	Acrinathrin, allethrin, biphenthrin, bioresmethrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerate, etofenprox, fenpropathrin, flucythrinate, fluvalinate, lambda-cyhalothrin, permethrin, piperonylbutoxide, tau-fluvalinate
Benzoylureas	Diflubenzurone, flucycloxurone, flufenoxurone, lufenurone, teflubenzurone, triflumurone
Others	Fipronyl, pyridabene, thiocyclam, thiodicarb, thiophanox
Systemic insecticides	
Organophosphorus compounds	Acephate, dimethoate, disulfotone, phorate, formothione, phosphamidone, heptenophos, methamidophos, mevinphos, thiometone, trichlorphon, vamidothion
Carbamates	Aldicarb, bendiocarb, benfuracarb, ethiophencarb, furathiocarb, carbofuran, carbosulfan, methomyl, oxamyl, pirimicarb
Others	Acetamiprid, bensultap, buprofezine, cyromazine, diaphenthiurone, hexaflumuron, imidacloprid, triazamate
Specific acaricides	
Without fungicide activities	
Organochlorin compounds	Chlorobenzilate, tetradifon
Organotin compounds	Cyhexatin
With fungicide activities	
Dinitro compounds	Binapicryl, dinocap
Molluscicides	
Aquatic	
Botanic	Endod
Others	Niclosamide, sodium pentachlorophenolate, tributyltin, triphenmorph, triphenyltin
Terrestrial	
Carbamates	Aminocarb, methiocarb, mexacarbate
Others	Metaldehyde
Non-systemic protective fungicides	
Dithiocarbamates	Maneb, mancozeb, metiram, propineb, thiram, zineb
Phthalimides	Carbendazim, dichlofluanide, folpet, captafol, captan
Dinitro compounds	Binapicryl
Organomercury compounds	Phenylmercury
Dicarboximides	Vinclozolin

(*continued overleaf*)

Table 12.60 (*continued*)

Pesticides	Names of pesticides representing a given group[a]
Organotin compounds	Fentin
Chlorinated aromatic compounds	Dichlone, diclorane, chlorothalonil, quintozene (PCNB), tecnazene (TCNB)
Cation-active tensides	Dodine, glyodine
Others	Iprodion, procymidone
Systemic curative fungicides	
Antibiotics	Blasticidine, cyclohexamide, kasugamycine, streptomycine
Benzimidazoles	Benomyl, thiabendazole, thiophanate-methyl
Morpholines	Dodemorph, tridemorph
Pyrimidines	Bupirimate, ethirimol
Piperazines	Triforine
Others	Metalaxyl, propiconazole, triadimefon
Herbicides applied to leaf	
Systemic or translocated	
Phosphonoamino acids	Gluphosinate, glyphosate
Benzoic acid derivatives	Dicamba, chlorophenprop-methyl, 2,3,6-TBA
Chlorinated aliphatic acids	Dalapon, TCA
Oxyphenoxyacid esters	Cycloxydim, diclofop-methyl, fenoxaprop-ethyl, fluazifop-butyl, haloxyfop-methyl, quizalofop-ethyl
Phenoxyalkanoic acids	2,4-D, 2,4-DB, dichlorprop, mecoprop, MCPA, MCPB, silvex, 2,4,5-T
Quaternary ammonium compounds	Diquat, paraquat
Applied to leaf, contact	
Benzonitriles	Bromoxynil, dichlobenil, ioxynil
Benzothiadiazoles	Bentazone
Carbanilates	Phenmedipham
Cyclohexenones	Cycloxydim, clethodim, sethoxydim
Dinitrophenols	Dinoseb
Diphenylethers	Acifluorfen, lactofen, nitrofen, oxyfluorfen
Applied to soil	
Acetanilides	Alachlor, butachlor, metolachlor, propachlor
Amides and anilides	Benzoylprop-ethyl, difenamide, naptalam, pronamide, propanil
Carbanilates and carbamates	Asulam, barban, bendiocarb, carbetamide, chlorpropham, propham, triallate
Dinitroanilines	Benephin, pendimethalin, trifluralin
Pyridazinones and pyridinones	Amitrol, dimethazone, fluridone, norflurazone, oxadiazone, pyrazone
Pyridineoxy- and picolinic acids	Fluroxypyr, clopyralid, picloram, triclopyr
Phenylureas or other substituted ureas	Dimefurone, diurone, fenurone, fluometurone, chlorbromuron, chlorotoluron, isoproturon, linuron, metobromuron, metoxuron, monolinuron, siduron

Table 12.60 (*continued*)

Pesticides	Names of pesticides representing a given group[a]
Sulfonylureas	Amidosulfuron, flupyrsulfuron-methyl, chlorimuron-ethyl, chlorsulfuron, metsulfuron-methyl, nicosulfuron, primisulfuron-methyl, prosulfuron, rimsulfuron, sulfometuron-methyl, sulfosulfuron, thiameturon-methyl, triasulfuron, tribenuron, triflusulfuron-methyl
Thiocarbamates	Butylate, cycloate, EPTC, molinate, pebulate, thiophencarb, triallate
Triazins	Ametryne, atrazine, desmetryne, hexazinone, cyanazine, methoprotryne, metribuzine, prometone, prometryne, propazine, simazine, terbumetone, terbuthylazine, terbutrizine, terbutryne
Uracils	Bromacil, lenacil, terbacil
Desiccants and defoliants	
Organophosphorus compounds	Merfos
Derivatives of phenols	Dinoseb
Quaternary ammonium compounds	Diquat, paraquat
Growth regulators and growth promoting substances	
Auxins	2,4-D, MCPB, NAD
Cytokinins	Adenine, kinetin
Gibberellins	GA_3, giban
Substances producing ethylene	Ethephon
Growth inhibitors and retardators	
Quaternary ammonium compounds	Chlormequate, mepiquate
Hydrazides	Daminozide, maleinhydrazide
Triazoles	Paclobutrazola, uniconazole
Rodenticides	
Fumigants and anticoagulants	
Hydroxycoumarins	Brodifacoum, difemacoum, coumafuryl, coumatetralyl, warfarin
Indanediones	Diphacinone, chlorophacinone, pindone
Without coagulation activities	
Arsenic compounds	Sodium arsenite, arsenic trioxide
Benzeneamines (anilines)	Bromethaline
Thioureas	Antu, promurit
Natural compounds	Red squill (*Drimia maritime*, Asparagaceae), strychnine
Others	Fluoroacetamide, sodium fluoroacetate, zinc phosphide, sodium norbormide

[a] Abbreviated names are derived from chemical or common names: DDT (dichlorodiphenyltrichloroethane), HCH (1.2.3.4.5.6-hexachlorocyclohexane), PCNB (pentachloronitrobenzene), TCNB (1,2,4,5-tetrachloro-3-nitrobenzene), 2,3,6-TBA (2,3,6-trichlorobenzoic acid), TCA (trichloroacetic acid), 2,4-D [(2,4-dichlorophenoxy)acetic acid], 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid], MCPA [(4-chloro-2-methylphenoxy)acetic acid)], MCPB [(4-chloro-o-tolyloxy)butyric acid)], 2,4,5-T (trolamine or 2,4,5-T-triethylammonium), EPTC (*S*-ethyldipropylthiocarbamate), GA_3 (gibberellic acid or gibberellin A_3).

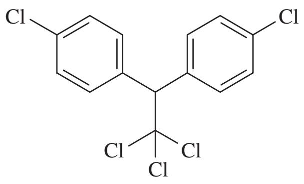
12.6.2.1.1 Persistent chlorinated hydrocarbons

Organochlorine pesticides are chlorinated hydrocarbons which were used extensively from the 1940s in agriculture and pest control. Most of these compounds were banned in 2004 when the Stockholm Convention went into effect. Measures to reduce or eliminate these persistent organic pollutants (POPs) were: elimination of their production and use (category A, which includes aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphene and also polychlorinated biphenyls and hexachlorobenzene, which also has use as an industrial chemical), and restriction and control of their production and use (category B, which includes DDT). Pentachlorophenol, formerly used as a herbicide, insecticide and fungicide, is now used mainly as a fungicide to protect wood or as a biocide in masonry. Its use as a herbicide is banned. Lindane is currently being examined at an international level and its worldwide ban is being considered.

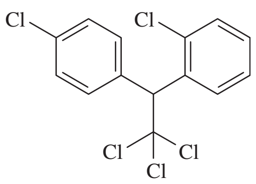
Organochlorine pesticides are mostly contact insecticides with neurotoxic effects that are characterised by their high lipophilicity and related high potential to accumulate in the adipose tissues of living organisms. These properties, along with high chemical stability and limited biodegradability, are the reasons why many organochlorine pesticides were banned, but their residues are still commonly found in the environment, either as the parent insecticides or some of their persistent metabolites. Some organochlorine pesticides are still registered for use in certain countries. The spectrum of the major contaminants in the food chain is due to their persistence. They are mainly present in fish and other aquatic animals, but also in tissues of higher mammals, including humans.

DDT

The insecticide DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane (technical grade DDT), was composed of up to 14 chemical compounds, of which only 65–80% was the active ingredient, *p,p′*-DDT (**12-150**). The other components included 15–21% of the nearly inactive isomer *o,p′*-DDT (**12-151**), up to 4%



12-150, *p,p′*-DDT



12-151, *o,p′*-DDT

of *p,p′*-DDD, dichlorodiphenyldichloroethane, up to 1.5% of 1-(4-chlorophenyl)-2,2,2-trichloroethanol, lower amounts of *o,p′*-DDD and closely related compounds with low insecticidal properties, *p,p′*- and *o,p′*-isomers of DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene. The physical and chemical properties of DDT and derived compounds are listed in Table 12.61.

DDT was first introduced in World War II (in 1940) as a very effective agent to wipe out malaria by killing the mosquitoes that carry the disease. Since that time, DDT, DDE and other components of technical grade DDT have entered the environment (Figure 12.54). DDT stays in the environment for long periods and can travel long distances from where it was originally used. Both compounds, DDT and DDE, have even been found in Arctic and Antarctic animals, even though DDT was never used in those regions. DDE also enters the environment as a DDT metabolite, which is produced by enzymatically catalysed biotransformation (dehydrochlorination) of DDT in living organisms. In adipose tissue of animals, the DDT dechlorination product DDD can also frequently be found. In air, DDT, DDE and DDD are rapidly broken down by sunlight (the estimated half-life is about 3 days), and they are sorbed strongly to soil. Most DDT in soil is broken down slowly to DDE and DDD by microorganisms; half the DDT in soil will break down in 2–15 years, depending on the type of soil. Biodegradation of DDT, for example, by white rot fungus *Phanerochaete chrysosporium* yields polar, water-soluble metabolites including DDD, FW-152 and 4,4′-dichlorobenzophenone (DBP) and 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (dicofol), one of the intermediates of DDT

Table 12.61 Physical and chemical properties of *p,p′*- and *o,p′*-isomers of DDT, DDE and DDD.

Property	*p,p′*-DDT	*o,p′*-DDT	*p,p′*-DDE	*o,p′*-DDE	*p,p′*-DDD	*o,p′*-DDD
Molecular weight	354.49	354.49	318.03	318.03	320.05	320.05
Melting point (°C)	109	74.2	89	-	109–110	76–78
Boiling point (°C)	Decomposes	-	336	-	350	-
Solubility in water at 25 °C (mg/l)	0.025	0.085	0.12	0.14	0.09	0.10
Vapour pressure (mPa)	2.1×10^{-8a}	1.5×10^{-8a}	880×10^{-8b}	83×10^{-8b}	818×10^{-8b}	826×10^{-8c}
log K_{ow}	6.91	6.79	6.51	6.00	6.02	5.87
log K_{OC}	5.18	5.35	4.70	5.19	5.18	5.19
k_H (Pa m^3/mol)	0.84	0.059	2.1	1.8	0.41	0.83

[a] At 20°C.
[b] At 25°C.
[c] At 30 °C.

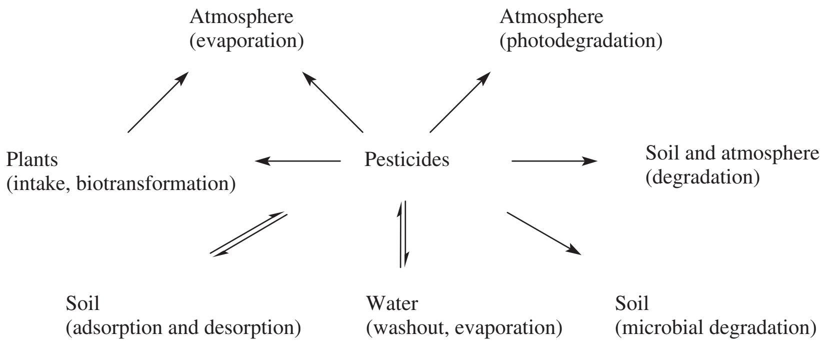


Figure 12.54 Fate of pesticides in the environment.

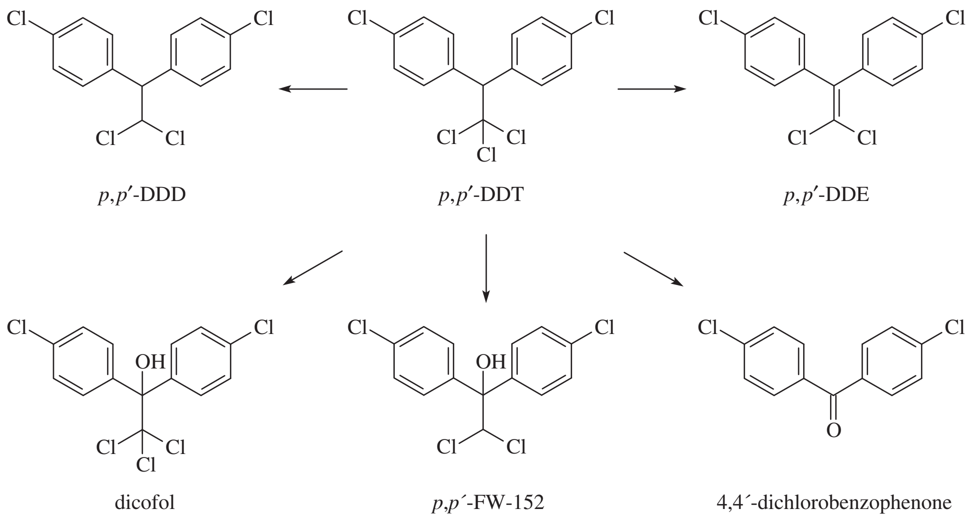


Figure 12.55 Transformation products of *p,p′*-DDT.

synthesis, effective against red spider mite, which was formerly used as a miticide (Figure 12.55).

Typical levels of DDT and derived compounds in different parts of the ecosystem in the mid-1970s can be seen in Figure 12.56. As a result of biomagnification, in some clams and fish, concentrations of DDT are ten times greater than those in the plankton, and the process of biomagnification goes up the food chain from one trophic level to the next. Gulls, which feed on clams, can accumulate 40 or more times the concentration of DDT that was in their prey. This represents a 400-fold increase in concentration along the length of this short food chain. The people applying the pesticides are exposed primarily to DDT, whereas nearly all of the general population is exposed to the metabolite DDE in the diet or drinking water. For example, in 1991, the IARC reported that the mean concentrations of DDT in the population have declined in much of the world: from 5–10 mg/kg to around 1.0 mg/kg of milk fat or even lower over the last three decades. DDT residues (residues of *p,p*'-DDT, *o,p*'-DDT, *p,p*'-DDE and *o,p*'-DDE) in human fat (1995–1997) in the UK were 1.0–9.3 mg/kg (47 cases), 0.1–0.9 mg/kg (135 cases) and 0.01–0.09 mg/kg (19 cases), which may reflect the measures taken since DDT was banned. DDE is mostly found in autopsy or biopsy samples of organisms, and also in the breast milk of mammals, often in much higher concentrations than the parent insecticide DDT. The ratio of these two substances (conversion rate) thus indicates the time from the primary organism load. The major metabolite detected in faeces and liver samples is *p,p′*-FW-152, 2,2-dichloro-1,1-bis(4-chlorophenyl)ethanol.

The amount of DDT in food has greatly decreased since DDT was banned, and should continue to decline. Between the years 1986 and 1991, the average adult in the United States, for example, consumed an average of 0.8 μg of DDT a day. Adults consumed slightly different amounts based on their age and sex. The largest fraction of DDT in a person's diet comes from meat, poultry, dairy products and fish. Leafy vegetables generally contain more DDT than other vegetables, possibly because DDT in the air is deposited on the leaves. Infants may be exposed by drinking breast milk. The changes of concentrations of DDT and related compounds during industrial and culinary processing of foods are minimal due to their relatively low volatility and considerable resistance to degradation. Health standards have laid down very strict criteria for DDT content in food. For example, in the EU the maximum concentration of DDT in drinking water is 0.1 μg/l, while the United States is more tolerant, with a limit set at 50 μg/l. Maximum residue

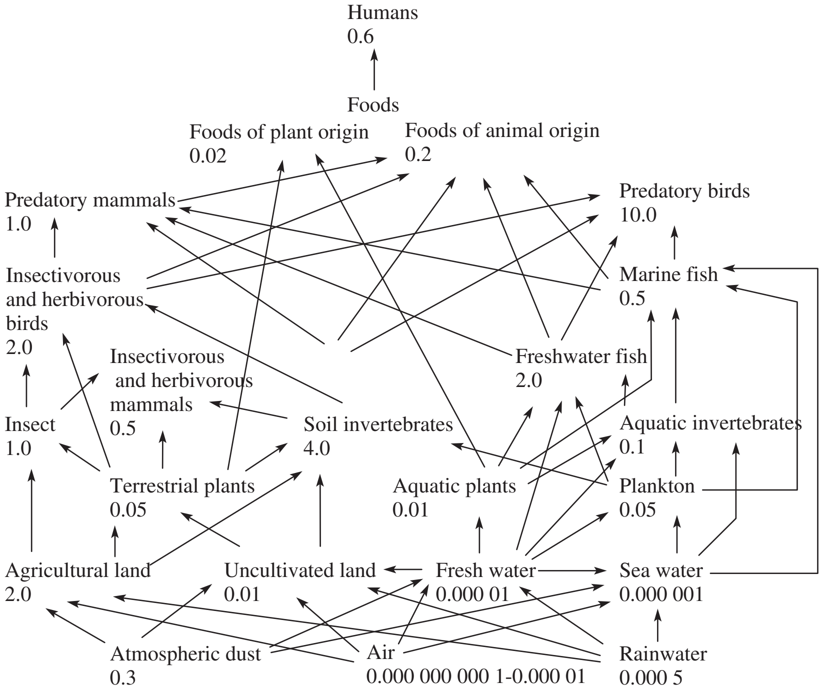


Figure 12.56 Typical levels (in mg/kg) of DDT and derived compounds in different parts of the ecosystem.

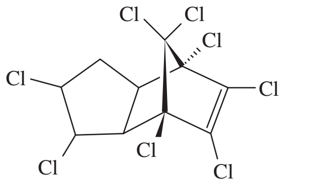
levels expressed as the sum of *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE and *o,p′*-DDE for fresh and frozen fruits and nuts are 0.05 mg/kg.

Potential mechanisms of DDT action on humans are genotoxicity and endocrine disruption. Acute toxicity is moderate, while chronic toxicity has been linked to diabetes. A number of studies have found a prevalence of diabetes in a population with increased serum levels of DDT or DDE. The IARC classified DDT as a Group 2B agent.[3]

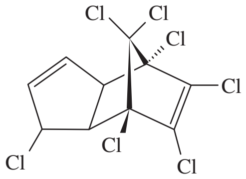
Other chlorinated hydrocarbons

From a toxicological point of view, the most hazardous chlorinated insecticides are cyclodienes known as **aldrin** and aldrin epoxide **dieldrin**, named after the Diels–Alder reaction, which was used for the synthesis of aldrin. Aldrin, 1,2,3,4,10,10-hexachloro-1, 4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene, was widely used until the 1970s, when it was banned in most countries. Dieldrin, chemical name 1a*R*,2*R*,2a*S*,3*S*,6*R*,6a*R*,7*S*,7a*S*)-3,4,5,6, 9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethano-naphtho[2,3-*b*]oxirane, is an extremely persistent insecticide that tends to biomagnify as it is passed along the food chain. It was widely used from the 1950s until the early 1970s. Dieldrin is also produced by photooxidation of aldrin, as is another product, photodieldrin, and a highly toxic stereoisomer of dieldrin known as **endrin** and certain other compounds (Figure 12.57). Endrin was primarily used as an insecticide and also as a rodenticide.

Chlordane, 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane (**12-152**), also known as chlorindane and chlorotox, is another cyclodiene produced by chlorinating cyclopentadiene to form hexachlorocyclopentadiene, and condensing the latter with cyclopentadiene. Chlordane is an insecticide used primarily in agriculture for the treatment of maize and citruses, and in



12-152, chlordane



12-153, heptachlor

households against termites. It was banned in the United States in 1988, but in some countries it is still used. Cyclodiene **heptachlor** (**12-153**), 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene is about 3–5 times more active as an insecticide, and is also produced via the Diels–Alder reaction of hexachlorocyclopentadiene and cyclopentadiene. This was used similarly to chlordane between the 1960s and 1970s in agriculture, but also in households as an insecticide to kill ants, termites and worms. It is still in use in some countries against ants and in the protection of underground electrical cables or around transformers. The use of heptachlor is now restricted to controlling fire ants in power transformers. Heptachlor is also a component of the insecticide chlordane. **Mirex**, 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1*H*-1,3, 4-(methanetriyl)cyclobuta[*cd*]pentalene (**12-154**), produced by the dimerisation of hexachlorocyclopentadiene in the presence of aluminium chloride, was used in agriculture to control ants and termites. In the 1960s, mirex was used as a flame retardant in plastics and building materials.

Another persistent chlorinated insecticide is **toxaphene**, a complex mixture of about 200 structurally related chlorinated terpenes (containing 6–11 chlorine atoms) derived from camphene by

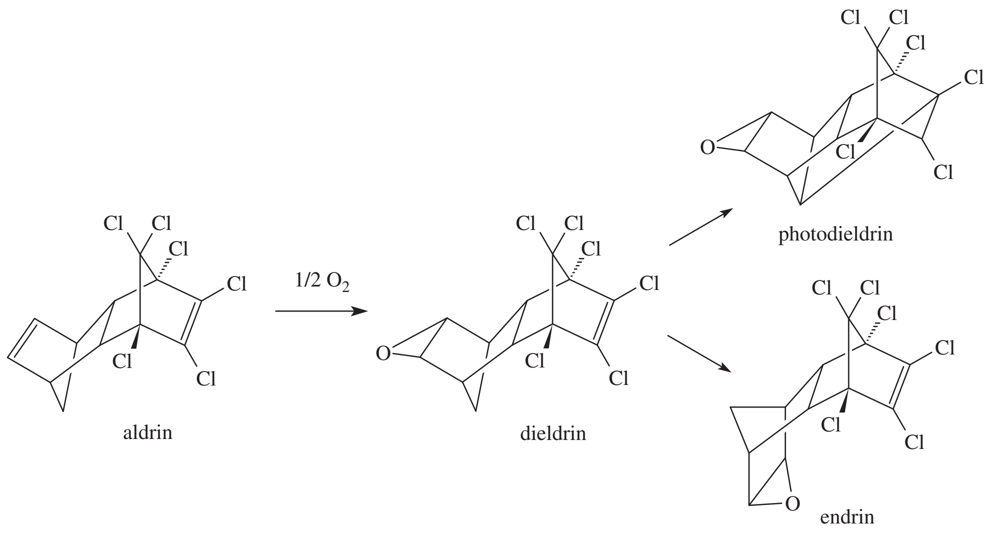
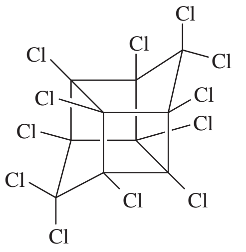


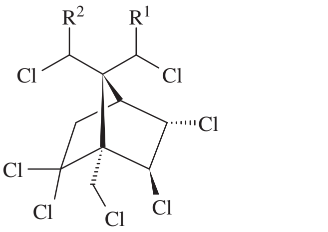
Figure 12.57 Transformation products of aldrin.



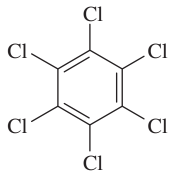
12-154, mirex

chlorination to an overall chlorine content of about 70%. Very little is known about the mode of action and metabolic fate of these compounds. They differ not only in their toxicities, but also in their stabilities in the environment. In technical preparations, octachlorobornanes known as toxicants A-1, A-2 and B (**12-155**) are predominant. For example, toxicant A-1 has the chemical name 2,2,5,6-tetrachloro-1,7-bis-chloromethyl-7-dichloromethylbicyclo[2.2.1]heptane. It was used mainly in the 1970s, and in 1982 it was banned for most situations, and in 1990 it was banned for all uses in the United States.

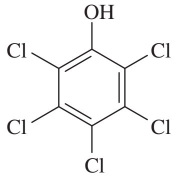
Important chlorinated insecticides of the group of alicyclic and aromatic chlorinated compounds are **hexachlorobenzene** (HCB), also known as perchlorobenzene or benzenehexachloride (BHC, **12-156**), **pentachlorophenol** (PCP, **12-157**) and **hexachlorocyclohexane** (HCH). Hexachlorobenzene is environmentally very dangerous, because due to its volatility, high stability and bioaccumulation it is able to spread over large distances. For example, similarly to DDT, it has been demonstrated in the air, water and tissues of animals and humans living in the Arctic region, although it was never used there. The technical product HCH is a mixture of eight stereoisomers, of which the most effective insecticide is γ-HCH, (1α,2α,3β,4α,5β,6β)-hexachlorocyclohexane, also called **lindane**, hexachloran or gammexan (**12-158**). This isomer is accompanied by α-HCH, which is (1α,2α, 3β,4α,5α,6β)-isomer, β-HCH (1α,2β,3α,4β,5α,6β)-isomer, δ-HCH (1α,2α,3α,4β,5α,6β)-isomer, ε-HCH, (1α,2α,3α, 4β,5β,6β)-isomer, ζ-HCH (1α, 2α,3α,4α,5α,6α)-isomer, η-HCH (1α,2α,3α,4α,5β,6β)-isomer and ξ-HCH (1α,2α,3α,4α,5α,6β)-isomer.



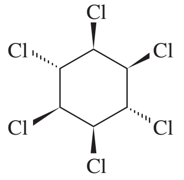
12-155, toxicant A-1, R^1 = Cl, R^2 = H
toxicant A-2, R^1 = H, R^2 = Cl
toxicant B, $R^1 = R^2$ = H



12-156, hexachlorobenzene



12-157, pentachlorophenol



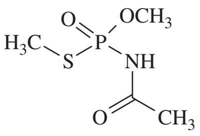
12-158, γ-1,2,3,4,5,6-hexachlorocyclohexane

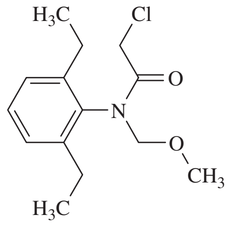
The IARC classified aldrin, dieldrin and endrin as Group 3 chemicals not classifiable as to their carcinogenicity to humans, but all the other chlorinated hydrocarbons (chlordane, heptachlor, mirex, toxaphene, hexachlorobenzene, pentachlorophenol and

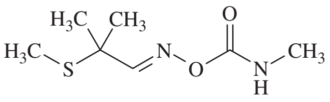
hexachlorocyclohexanes) were classified as Group 2B compounds, probably carcinogenic to humans.[3]

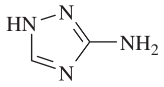
12.6.2.1.2 Modern pesticides

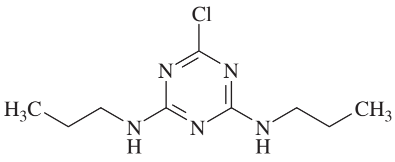
Table 12.62 gives some of the physico-chemical characteristics of modern pesticides. Structures of some of these pesticides are given in formulae **12-159** to **12-200**. Commonly used compounds were selected, residues of which have been and still are found in foods relatively frequently. In comparison with the previous group of persistent organochlorine compounds, modern pesticides are mostly substances that have higher solublity in water and lower pK_{OW} values.

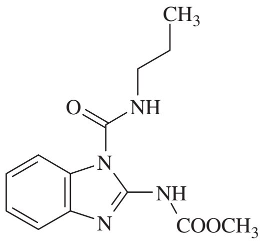

12-159, acephate

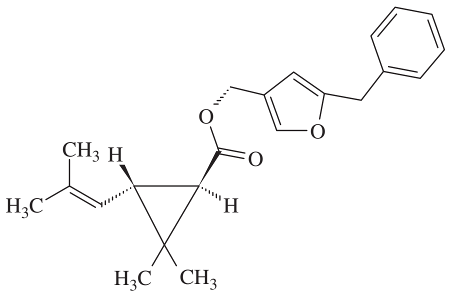

12-160, alachlor

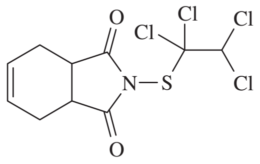

12-161, aldicarb

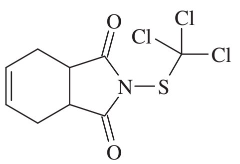

12-162, amitrol

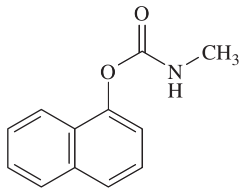

12-163, atrazine

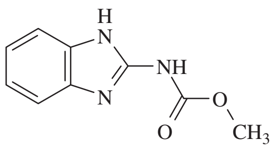

12-164, benomyl

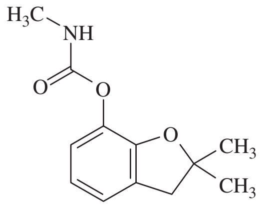

12-165, bioresmethrin

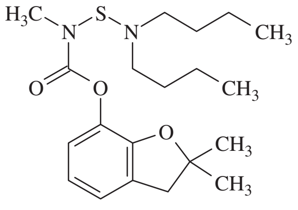

12-166, captafol

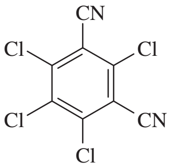

12-167, captan

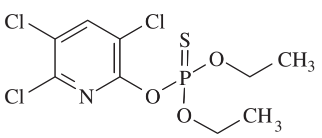

12-168, carbaryl

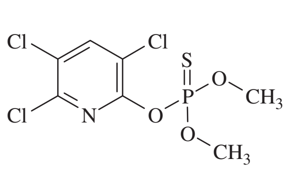

12-169, carbendazim

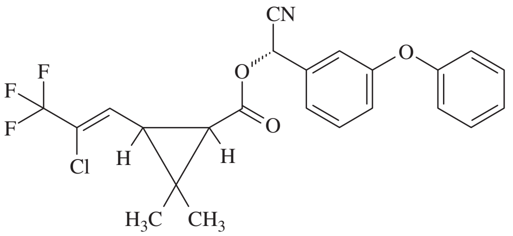

12-170, carbofuran

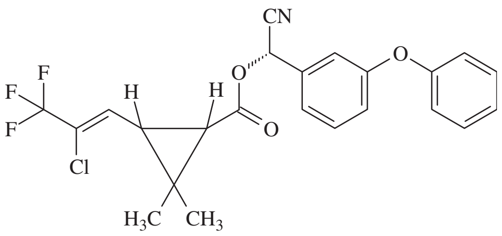

12-171, carbosulfan

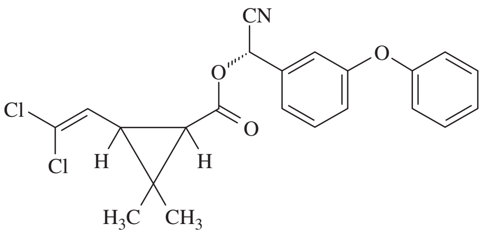

12-172, chlorothalonil


12-173, chlorpyrifos

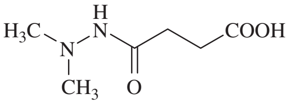

12-174, chlorpyrifos-methyl


12-175, cyhalothrin, (*S*)-alcohol (*Z*)-(1*R*)-*cis*-acid

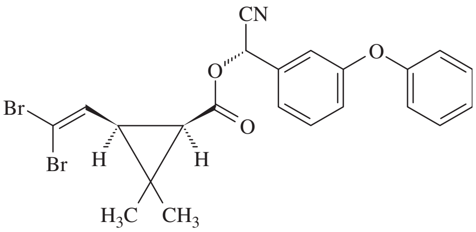

12-176, cyhalothrin, (*R*)-alcohol (*Z*)-(1*R*)-*cis*-acid



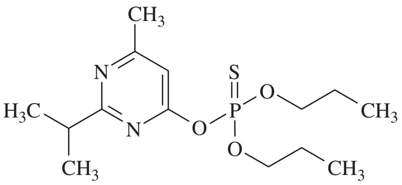
12-177, cypermethrin, (*S*)-alcohol (1*R*)-*cis*-acid racemate comprising (*R*)- and (*S*)-α-cyano-3-phenoxybenzyl (1*S*) and (1*R*)-*cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate



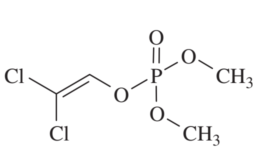
12-178, daminozide



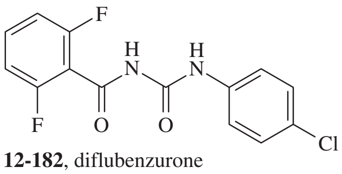
12-179, deltamethrin, (*S*)-alcohol (1*R*)-*cis*-acid



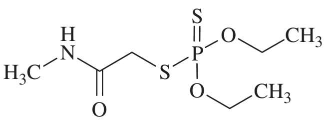
12-180, diazinone



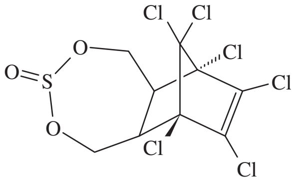
12-181, dichlorvos



12-182, diflubenzurone



12-183, dimethoate



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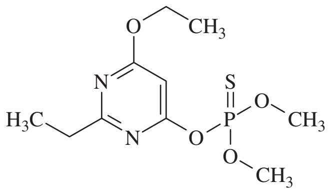

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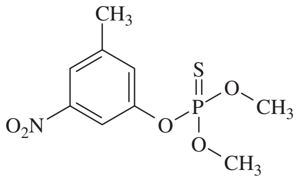
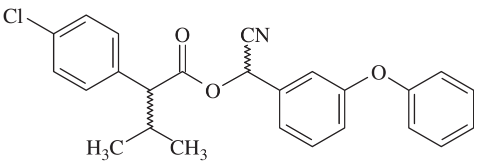
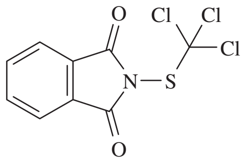


12-186, fenitrothione



12-187, fenvalerate



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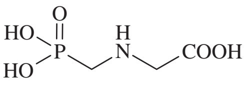
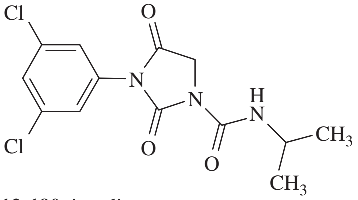
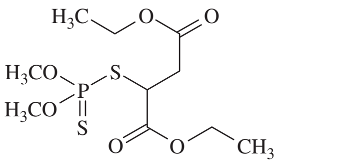


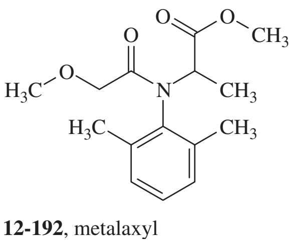
12-189, glyphosate



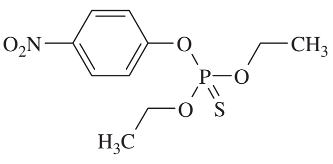
12-190, iprodion



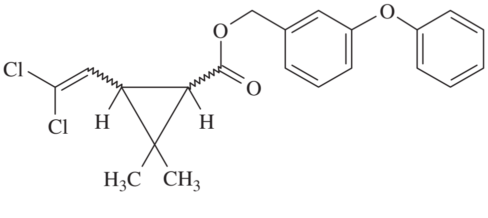
12-191, malathione



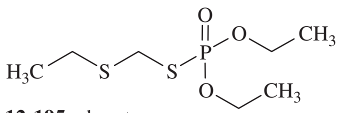
12-192, metalaxyl



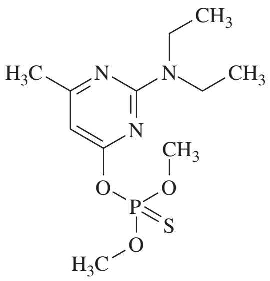
12-193, parathione



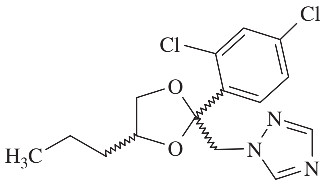
12-194, permethrin



12-195, phorate



12-196, pirimifos-methyl



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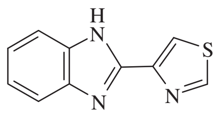
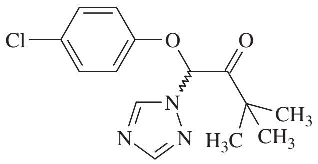
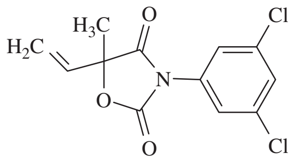


12-198, thiabendazole



12-199, triadimefon



12-200, vinclozolin

Table 12.62 Important physical and chemical properties of modern pesticide representatives.

Common name	Chemical name	Solubility in water (mg/l)[a]	Saturated vapour pressure (mPa)[a]	pK_{ow}
Acephate	*O,S*-Dimethylacetylphosphoramidothioate	790 000 (20)	0.226 (24)	−0.89
Alachlor	2-Chloro-2′,6′-diethyl-*N*-methoxymethylacetanilide	242 (25)	2.1 (25)	3.09
Aldicarb	2-Methyl-2-(methylthio)propionaldehyde-*O*-methylcarbamoyloxime	4.9 (20)	13 (20)	0.05
Amitrol	1*H*-1,2,4-Triazol-3-ylamine	280 000 (23)	0.000 33 (20)	−0.97
Atrazine	6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	33 (22)	0.0385 (25)	2.5
Benomyl	Methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate	4 (25)	<0.005 (25)	1.37
Bioresmethrin	(1*R*,3*R*)-5-Benzyl-3-furylmethyl-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate	<0.3 (25)	18.6 (25)	>4.7
Captafol	*N*-(1,1,2,2-Tetrachloroethylthio)cyclohex-4-en-1,2-dicarboximide	1.4 (20)	– [b]	3.8
Captan	*N*-(Trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide	3.3 (25)	<1.3 (25)	2.79
Carbaryl	1-Naphthylmethylcarbamate	120 (20)	0.041 (23.5)	1.59
Carbendazim	Methyl benzimidazol-2-ylcarbamate	29 (24)[c]	0.09 (20)	1.38[d]
Carbofuran	2,2-Dihydro-2,2-dimethylbenzofuran-7-yl-methylcarbamate	351 (25)	0.031 (20)	1.52
Carbosulfan	2,3-Dihydro-2,2-dimethylbenzofuran-7-yl-(dibutylaminothio)methylcarbamate	0.3 (25)	0.041 (25)	–
Chlorothalonil	2,4,5,6-Tetrachloro-1,3-benzenedicarbonitrile	0.81 (25)	0.076 (25)	2.89
Chlorpyrifos	*O,O*-Diethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate	0.4 (25)	2.7 (25)	4.70
Chlorpyrifosmethyl	*O,O*-Dimethyl-*O*-3,5,6-trichloro-2-pyridylphosphorothioate	2.6 (20)	3 (25)	4.24
Cyhalothrin	(*RS*)-α-Cyano-3-phenoxybenzyl-(*Z*)-(1*RS*,3*RS*)-2-chloro-3,3,3-trifluorpropenyl)-2,2-dimethylcyclopropancarboxylate	0.000 006 (20)	0.001 (20)	6.8

Table 12.62 (*continued*)

Common name	Chemical name	Solubility in water (mg/l)[a]	Saturated vapour pressure (mPa)[a]	pK_{ow}
Cypermethrin	(*RS*)-α-Cyano-3-phenoxybenzyl-(1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	0.004	0.000 23 (20)	6.6
Daminozide	*N*-Dimethylaminosuccinamic acid	100 000 (25)	22.7 (23)	−1.5
Deltamethrin	(*S*)-α-Cyano-3-phenoxybenzyl-(1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate	<0.0002 (25)	0.000 012 (25)	4.6
Diazinone	*O*,*O*-Diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)phosphorothioate	60 (20)	12 (25)	3.3
Dichlorvos	2,2-Dichlorovinyldimethyl phosphate	8000 (25)	2100 (25)	1.9
Diflubenzurone	1-(4-Chlorophenyl)-3-(2,6-difluorobenzoyl)urea	0.08 (25)	0.001 2 (25)	3.89
Dimethoate	*O*,*O*-Dimethyl-*S*-methylcarbamoylmethylphosphorodithioate	230 (20)	0.25 (25)	0.70
Endosulfan	(1,4,5,6,7,7-Hexachloro-8,9,10-trinorborn-5-en-2,3-ylenebismethylene)sulfite	0.32 (22)	0.83 (20)	4.74
Etrimfos	*O*-6-Ethoxy-2-ethylpyrimidin-4-yl-*O*,*O*-dimethylphosphorothioate	40 (23)	6.5 (20)	3.3
Fenitrothione	*O*,*O*-Dimethyl-*O*-(4-nitro-*m*-tolyl)phosphorothioate	21 (20)	15 (20)	3.43
Fenvalerate	(*RS*)-α-Cyano-3-phenoxybenzyl-(*RS*)-2-(4-chlorophenyl)-3-methylbutyrate	<0.01 (25)	0.019 (20)	5.01
Folpet	*N*-(Trichloromethylthio)phthalimide	1 (20)	0.021 (25)	3.11
Glyphosate	*N*-(Phosphonomethyl)glycine	11 600 (25)	–[b]	−3.4
Iprodion	3-(3,5-Dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide	13 (20)	0.000 5 (25)	3.0[d]
Malathione	Diethyl (dimethoxythiophosphorylthio)succinate	145 (25)	5.3 (30)	2.75
Metalaxyl	Methyl-*N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate	8400 (22)	0.75	1.75
Parathione	*O*,*O*-Diethyl-*O*-4-nitrophenylphosphorothioate	11 (20)	0.89 (20)	3.83
Permethrin	3-Phenoxybenzyl-(1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	0.2 (20)	0.07 (20)	6.10
Phorate	*O*,*O*-Diethyl-*S*-ethylthiomethylphosphorodithioate	50 (25)	85 (25)	3.92
Pirimifosmethyl	*O*,*O*-Dimethyl-*O*-2-diethylamino-6-methylpyrimidin-4-ylphosphorothioate	9.9 (30)[d]	2 (20)	4.20
Propiconazole	(±)-1-[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole	100 (20)	0.056 (25)	3.72
Thiabendazole	2-(Thiazol-4-yl)benzimidazole	<0.05[d]	–[b]	
Triadimefon	1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one	64 (20)	0.02 (20)	3.11
Vinclozolin	(*RS*)-3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione	2.6 (20)	0.13 (20)	3.00

[a]The numbers in parentheses = temperature in °C.
[b]Negligible at normal temperature.
[c]At pH 4.
[d]At pH 5.

12.6.2.2 Biological activity

The inhibition or elimination of harmful factors by the use of pesticides includes a wide range of mechanisms. Their detailed specification is not possible, therefore only some illustrative examples are listed.

The basis of insecticidal effects typical of classical organochlorine compounds, such as DDT, and pyrethrins, such as pyrethrin I and the synthetic pyrethroids, organophosphates or carbamates, is their neurotoxicity, but the mechanism of action is not identical for all compounds. In insects DDT and pyrethrins act on the voltage-gated sodium channel by prolonging the inactivation current. Other chlorinated hydrocarbons, including lindane and octachlorobornane toxicant A-2 (the principal active ingredients of hexachlorocyclohexane and toxaphene) and the cyclodienes are neurotoxicants with a different target that was finally defined as the heteropentameric γ-aminobutyric acid (GABA)-gated chloride channel. Organophosphates and carbamates, such as carbaryl, ethephon, dichlorvos, dimethoate and acephate, inhibit the enzyme acetylcholine esterase (they act as serine hydrolase inhibitors) by phosphorylation or carbamoylation of the bound serine hydroxyl group in the active centre of the enzyme (acetylcholine is the only neurotransmitter employed in the motor division of the somatic nervous system and the principal neurotransmitter in all autonomic ganglia). Insecticidal benzoylureas, by contrast, are inhibitors of chitin synthesis, and thus inhibit the construction of insect cuticles. The mechanism of action of fungicides is actually heterogeneous as the attack targets may be the fungal hyphae and their spores. For example, the fungitoxic effect of widely used ethylenebisdithiocarbamates (EBDC) or phthalimides is based, among other things, on the inhibition of enzymes containing sulfhydryl groups. The toxicity of another important group of fungicides, benzimidazoles, is based on their interference with the biosynthesis of DNA (its replication during cell division). Herbicidal effects are based on an extraordinarily diverse range of mechanisms. For example, phenoxyalkanoic acids and derivatives of benzoic acids cause an atypical growth of sensitive plants (targeted weeds), which is based on disorders of nucleic acid metabolism caused by herbicide applied on the leaves, which is similar to the effect of plant hormones or plant growth regulators termed auxins. Total (non-selective) herbicides from the group of quaternary (bipyridylium) ammonium compounds interfere with the process of photosynthesis, which is mainly due to irreversible changes in cell membranes, in which they catalyse the oxidation of fatty acids. The effect of nitroaniline is based on retardation of weed seed germination, inhibition of root development and cessation of mitosis. Herbicides, such as triazines, phenylureas or uracils inhibit electron transport during photosynthesis (the Hill reaction in chloroplasts). The Hill reaction is formally defined as the reduction of an electron acceptor by electrons and protons from water, with the evolution of oxygen, when chloroplasts are exposed to light.

12.6.3 Occurrence and main sources

12.6.3.1 Release into the environment

With the exception of certain fumigants, pesticides are rarely used as pure compounds. In addition to the active ingredients, commercial preparations (formulations) contain various other components (solvents, emulsifiers and adhesives) that facilitate or improve the storage, handling and biological effects of pesticide formulations in the period after application. The treatment of crops (or livestock) is done by pesticide formulations in the form of sprays, aerosols, powders or granules. Unlike other groups of environmental contaminants, the entry of pesticides into the environment should be under controlled conditions and in accordance with the principles of good agricultural practice.

Conditions under which pesticides are used (with regards to data of producers, these are approved at the national level) must ensure effective and reliable pest control, but the quantity and method of use must also ensure that pesticide residues in treated products are minimal. Pesticides are often applied to the leaf surface, however the target organism is not necessarily the plant itself, but parasitic fungi or insects. Pesticides with **systemic effects** penetrate through the leaf cuticle and are translocated within the plant. The mobility of substances with **quasi-systemic effects** (some fungicides and insecticides) is lower and only a limited amount of the active agent penetrates the cuticle. The third category of substances are called **contact pesticides**, which exhibit a local effect only in places where their surface deposits are found. Pesticides may also be applied directly to the soil or get there during the crop treatment, which accounts for about 35–50% of the pesticide used, depending on the type of plant and density of vegetation. Systemic pesticides in soil are taken up by the roots and transported to the aerial parts of the plant. Availability of the pesticide from the soil is mainly determined by its solubility and the organic carbon content of the soil (due to the possibility of its immobilisation by sorption), and for non-polar compounds is inversely proportional to the value of K_{OC}.

Application of pesticide formulations, in particular in the form of sprays and powders, can lead to significant contamination of the atmosphere. On average, 10–20% of the pesticide used is thus in the form of vapour or droplets associated with the solids and transported by air flow to more or less remote locations, which may cause the pollution of agroecosystems. Pesticides with higher vapour pressure may be re-evaporated from the terrestrial or aquatic environment. Long-range transport of residues is particularly relevant in the case of persistent organochlorine compounds. After application, pesticides are affected by several chemical, physical and biological factors. Under field conditions, these factors can be applied individually or simultaneously depending on the type of application and the properties of the active substance. In general, pesticides used today, which are classified as modern, are characterised with respect to their physico-chemical properties, by

short-to-medium persistence and accumulation in the food chain is low to negligible. Insecticides, herbicides and fungicides to which harvested crops were exposed during the growing season have **preharvest withdrawal times** (the minimum interval between the last application and harvest) and following label directions will ensure that residues of these pesticides either do not remain on the plant material or remain there in amounts that do not exceed the maximum residue limits (MRLs) when crops are harvested, eaten or fed to livestock.

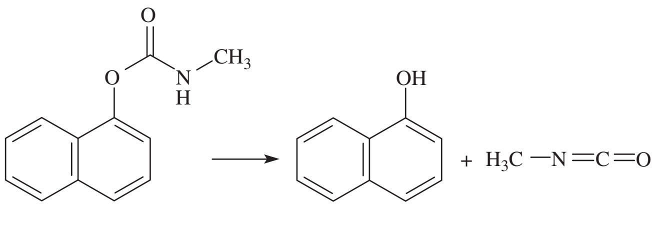


Figure 12.59 Thermal decomposition of carbaryl

12.6.3.1.1 Degradation in abiotic environment

Physical factors applied during the degradation of pesticides are mostly the energy from light and heat. In particular, photolysis of residues (occurring on the surface of leaves, in soil, atmosphere or in the aquatic environment is one of the most important processes leading to elimination of a number of pesticides from the environment. Along with singlet oxygen (see Section 3.8.1.8.4), reactive hydroxyl and superoxide radicals, and other free radicals generated by photochemical reactions, play an important role. Direct photolysis by solar radiation is involved only in part of the photodegradation processes. An example of these reactions is photolysis of aldrin (Figure 12.57).

An important reaction to which pesticides are subjected in the environment is hydrolysis, which is especially rapid at extreme pH values. As an example, hydrolysis of the synthetic pyrethroid insecticide permethrin to isomers of dichlorovinyl derivatives of chrysanthemic acid and 3-phenoxybenzyl alcohol (Figure 12.58) may be mentioned. A relatively rapid hydrolytic cleavage of ester bonds also occurs in many organophosphates. Examples of pesticides that are easily degraded by heating are *N*-methyl carbamates. For example, carbaryl is degraded to naphthalen-1-ol (1-naphthol) and methylisocyanate (Figure 12.59). Decomposition of fungicide benomyl analogously yields butylisocyanate and carbendazim, which has similar effects as benomyl. Carbendazim is slowly hydrolysed and decarboxylated to 2-aminobenzimidazole. Decarboxylation of butylcarbamic acid produces butylamine as a minor product, which reacts with butylisocyanate to yield *N*,*N*′-dibutylurea (Figure 12.60). Of course, the ease of decomposition of pesticides is also reflected in the length of the withdrawal period.

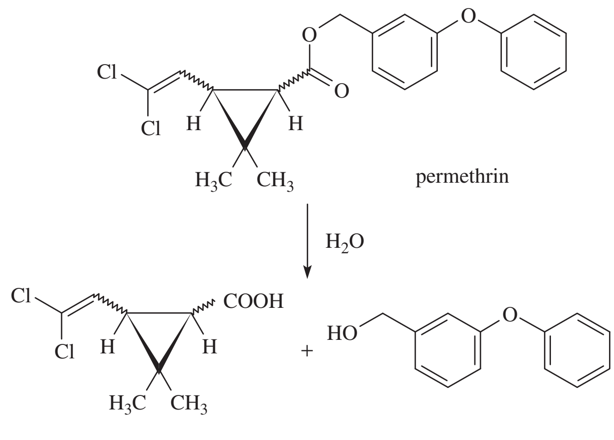


Figure 12.58 Hydrolysis of permethrin.

12.6.3.1.2 Degradation in biotic environment

Biological factors involved in the degradation of pesticides in soil and aqueous environments include particular microorganisms, various bacteria, fungi and actinomycetes. In principle, there are two types of degradative processes:

- **co-metabolism**, when the pesticide biotransformation proceeds by normal metabolic processes in a microorganism that is incapable of using the substrate as a source of energy or building material (the organism is actually growing on a second substrate and is transforming the pesticide without gaining the benefit);

- **catabolism**, when the pesticide becomes a substrate for a given microorganism (a source of carbon or nitrogen), adaptations can be found especially in bacteria during repeated exposure to xenobiotics.

Biodegradation of pesticides, however, also takes place in exposed plants and animals, whether they are target or non-target organisms, and the formed products can reside in the human food chain. In particular, vertebrates (especially birds and mammals) have active enzyme systems capable of effective degradation of xenobiotics. A brief description of these processes is presented in the following discussions, along with some specific examples.

Activation of protective mechanisms occurs when the harmful substance penetrates into the body. The penetration rate of pollutants and their distribution in the body, however, as well as the range of detoxification or elimination mechanisms, is subject to anatomical, physiological, biochemical and other factors. Phase I biotransformation typically involves changes catalysed by hydrolases and oxidases, in which polar functional groups are

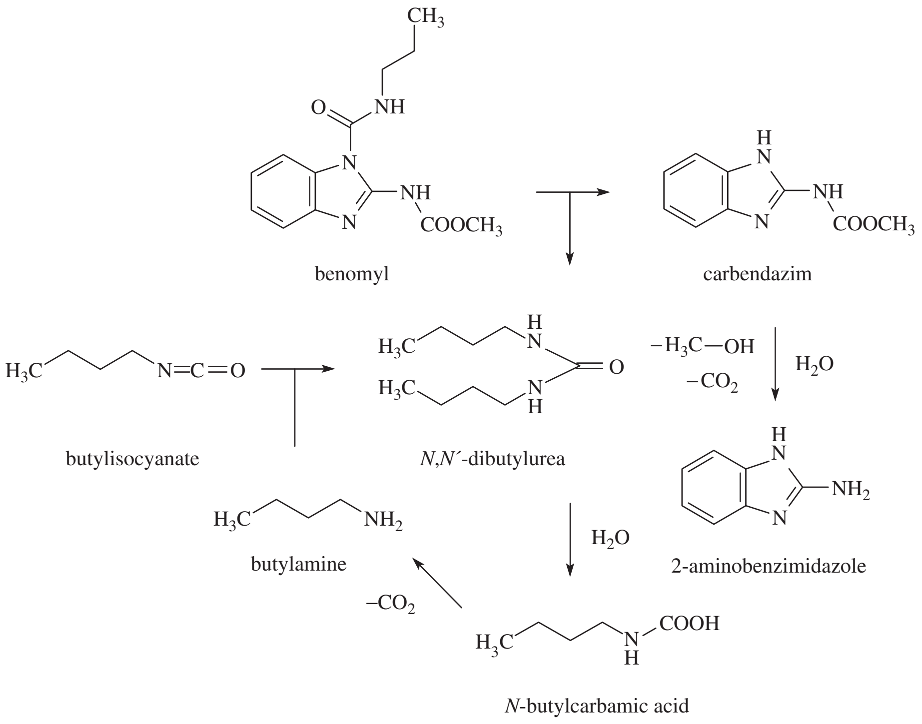


Figure 12.60 Decomposition of benomyl.

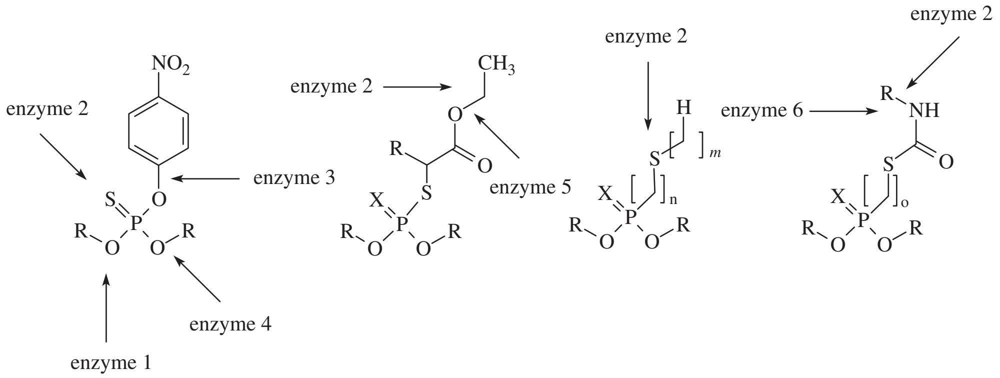


Figure 12.61 Enzymes involved in the biotransformation of organophosphorus compounds.

introduced to the molecule of the parent compounds, or these groups are formed from the parent molecule by degradation. For the example of organophosphates, Figure 12.61 shows the places of enzymatic attack in the initial phase of the biotransformation. The resulting primary metabolites often additionally enter into secondary reactions of phase II, which leads to their conjugation with small polar endogenous molecules to produce products that can be easily excluded from the organism. The type of resulting secondary metabolites is characteristic of each species. For example, in plants and invertebrates conjugation with D-glucose predominates. In plants, the waste products are apparently stored in lignin structures. In mammals, birds and certain fish, conjugation with D-glucuronic acid and reduced glutathione (GSH) primarily takes place, while in other fish conjugation with glycine occurs too. The metabolites in animals are transported by blood and excreted.

The complexity of degradation processes that take place in the biotic environment is demonstrated by a widely used herbicide atrazine (Figure 12.62). Substances of this group (symmetrical 1,3,5-chlorotriazines known as *s*-chlorotriazines) may be hydrolysed non-enzymatically to hydroxytriazines, which no longer exhibit herbicidal activity. Cleavage of a chlorine atom from the carbon C-2 is catalysed by the naturally occurring benzoxazinoid hydroxamic acids, such as (*R*)-2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, which is known under the acronym DIMBOA (see Figure 10.12). Together with other derivatives, glucoside of this benzoxazinoid hydroxamic acid is present in plants tolerant to triazines, such as maize, wheat, rye and other monocotyledonous plants. Alternative detoxification of atrazine is an enzymatically catalysed conjugation with glutathione. Resulting products are also inactive as herbicides. As is evident from

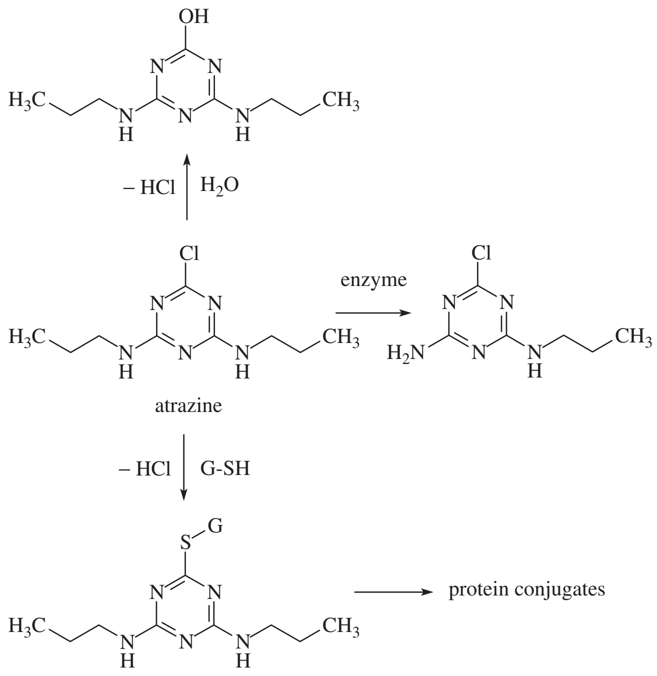


Figure 12.62 Biodegradation of atrazine (G-SH = reduced glutathione, enzyme = microsomal monoxidase).

Figure 12.62, another possibility of atrazine biodegradation may be dealkylation on the secondary amino group at carbons C-4 and C-6. Dealkylated metabolites partly retain the herbicidal activity of the parent compound.

Figure 12.63 provides another example of the diversity of metabolic processes to which pesticides are subjected after application. In the case of the carbamate insecticide carbaryl, the predominant processes are oxidation and hydrolysis, which may be followed by conjugation of primary metabolites with glutathione. The character of metabolic transformations is closely related to the pesticide selectivity (toxicity) to target and non-target organisms.

12.6.3.2 Contamination of foods

Transformation processes generally have a detoxifying nature, but can also lead to the formation of products that show more or less similar toxic effects on the target harmful organisms and on humans. Examples include formation of dicofol from DDT (Figure 12.55), carbendazim from benomyl (Figure 12.60) or aldoxycarb from aldicarb (Figure 12.64). In some cases, the formed substances can even be significantly more toxic than the parent compound. For example, the *in vivo* activation (desulfuration) of the insecticide parathione leads to the formation of paraoxone (Figure 12.65), the active form of the insecticide and a very potent inhibitor of acetylcholinesterase. A typical pro-insecticide is carbosulfan, which is hydrolysed in the insect body to biologically active carbofuran (Figure 12.66). In mammals, this reaction does not take place (the case of a highly selective compound), which explains the very low acute toxicity of carbosulfan for mammalian organisms.

Toxic compounds may likewise be produced from relatively non-toxic precursors during storage or handling of contaminated materials. Probably the most significant example is the formation of ethylenethiourea (ETU), which is one of many degradation products of extensively used fungicides known as ethylenebisdithiocarbamates (EBDCs, Figure 12.67). According to the IARC, ETU is not classifiable as to its carcinogenicity to humans. In the late 1980s, special attention was devoted to the formation of the *N*,*N*-dimethylhydrazine from the popular growth regulator daminozide used for fruit ripening, which is a potential carcinogen of Group 2B (Figure 12.68). Phenoxyalkanoic acids can yield relatively persistent chlorinated phenols as decomposition products, which can accumulate in the human food chain. Chlorinated phenols also arise from other precursors, for example from the pesticides lindane and pentachlorophenol.

12.6.3.2.1 Influence of technological and culinary operations

The operations used in the processing of farm crops or domestic culinary procedures usually have a significant impact on the residue levels in the final products. Generally the following can occur:

- a decrease in the level of pesticide residue due to pesticide degradation, volatilisation or selection of the edible portion with lower pesticide content;
- concentration of the pesticide residue in the edible portion due to the uneven distribution of residues in the commodity or higher affinity of the pesticide to the edible portion;
- formation of toxic degradation products from relatively non-toxic precursors.

Information on the effects that cause an increase or decrease in levels of pesticide residues in food raw materials and the final products are very important, because in estimating health risks from dietary exposure to residues of pesticides, it is necessary to consider the form in which the given food is consumed. In most cases, technological and culinary processes lead to a reduction, and often to the complete removal, of pesticide residues.

Drying

Under certain circumstances, drying may instigate an increase in pesticide concentration caused by an increase in the dry matter content. The level of volatile pesticides, on the other hand, decreases in the surface of the product. Drying in the sun can also cause photolysis of some pollutants. Freeze-drying does not usually result in lower levels of pesticide residues.

Removal of surface layers

Removal of surface layers may, in crops such as bananas, pineapples, kiwi, citruses, melons and others, result in a marked reduction in the levels of non-systemic pesticide residues applied directly to the crop

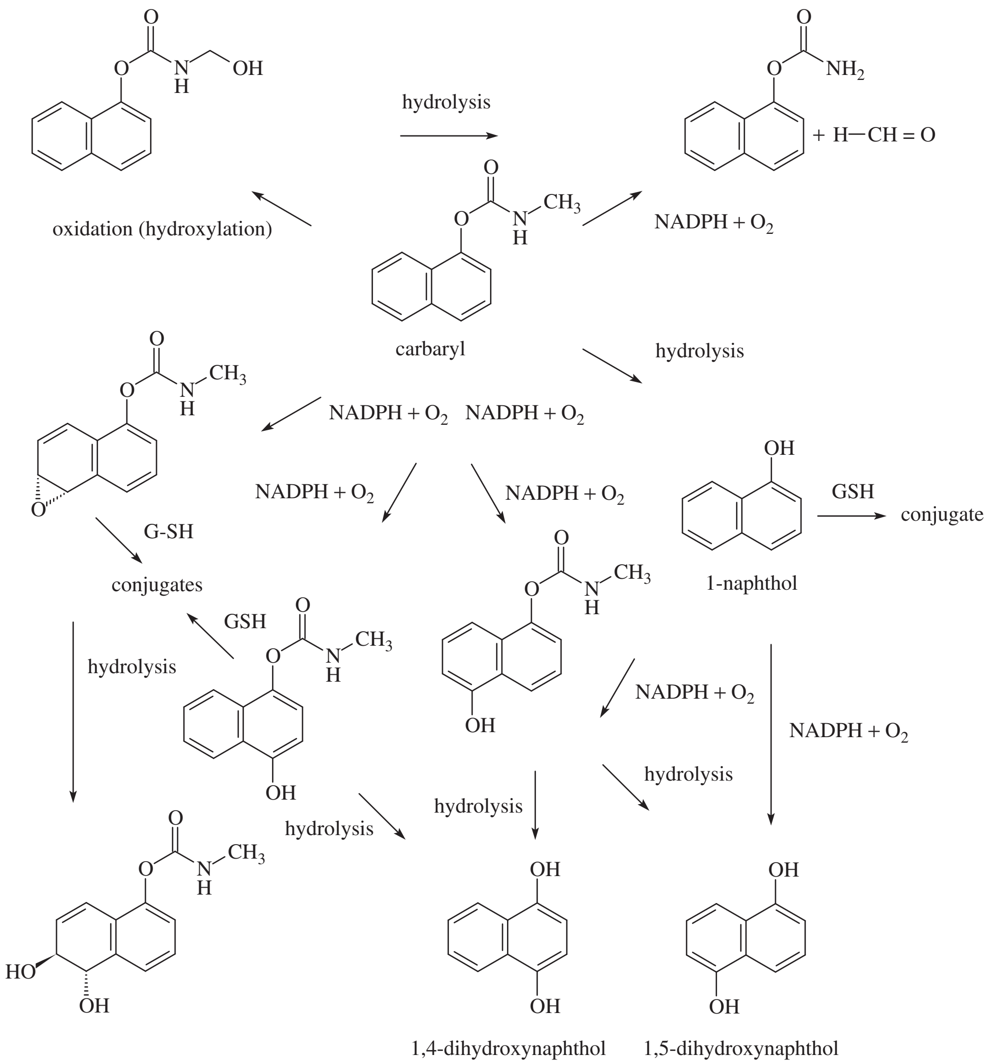


Figure 12.63 Biodegradation of carbaryl (G-SH = reduced glutathione).

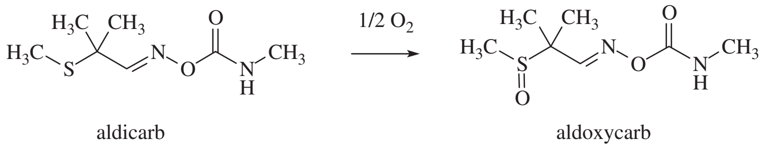


Figure 12.64 Transformation of aldicarb to aldoxycarb.

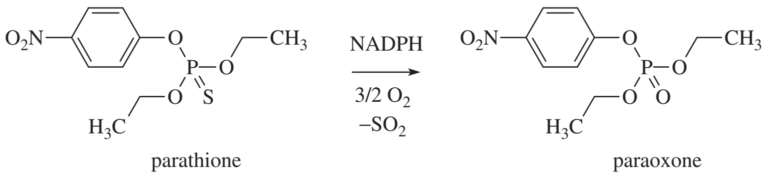


Figure 12.65 Transformation of parathione to paraoxone.

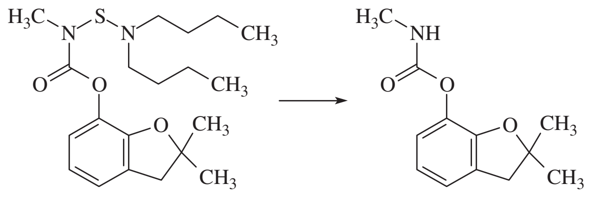


Figure 12.66 Transformation of carbosulfan to carbofuran.

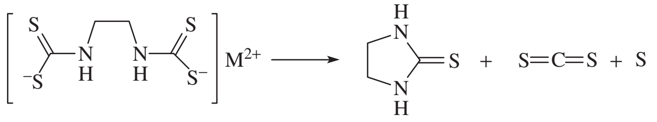


Figure 12.67 Transformation of ethylenebisdithiocarbamates to ethylenethiourea.

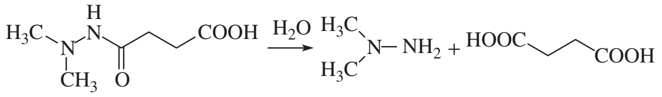


Figure 12.68 Transformation of daminozide into *N*,*N*-dimethylhydrazine.

before or after harvest. A similar effect can be achieved by removing the surface leaves of some vegetables (such as cabbage) and the surface layers of potatoes or cereals. For example, decontamination of potatoes grown in soil containing phorate was accomplished by peeling, which reduced the residue levels by 30%. Removing the outer husks of the rice grain treated with insecticide based on pirimiphos-methyl (to obtain brown rice) and, especially, removing the bran (the rest of the husk and the germ to obtain white rice), may lower the pesticide residue level to 70–90% of the original content. In the case of systemic pesticides, however, this process does not lead to product decontamination. Feeding livestock contaminated waste often leads to inadvertent infiltration of pesticide residues into the human food chain, especially in the case of lipophilic pesticides that may bioaccumulate.

Washing and blanching

Most of the technological processes used in fruit and vegetable processing include washing and blanching. The decrease in the levels of pesticide residues is possible particularly in the case of pesticides that are soluble in water, or in the case of contact pesticides deposited on the plant surface that can be mechanically removed. The overall effects resulting in a reduction of pesticide residues depend on a number of factors, the most important of which are:

- localisation of residues – only residues on the surface can be removed and not systemic compounds;
- age of residue – after application to the surface of the crop, pesticides gradually penetrate into the deeper layers of the cuticle and cannot, therefore, be easily removed later;
- solubility in water – increased lipophilicity of residues increases their affinity for cuticular waxes and washing is not very effective (lipophilic insecticides such as parathione, fenitrothione and diazinone, for example, are relatively difficult to remove);
- temperature and type of bath – a higher temperature or the addition of a detergent will solubilise residues, meaning they can be better removed from the surface; in an alkaline bath (containing sodium hydroxide), sometimes used to facilitate peeling of fruits and vegetables, the level of pesticides can be significantly reduced or even eliminated.

Boiling

Hydrothermal processes lead to a large reduction of residue levels, which is associated not only with volatile substances that vaporise with water vapour, but also with elevated temperatures that accelerate the hydrolysis of pesticides. For example, the loss of malathione can reach almost 100% in cooked spinach and 92% in cooked rice, while cooking tomatoes contaminated with carbaryl can decrease the pesticide concentration to 31%, and cooking meat contaminated with organophosphate insecticides similarly has a decontaminating effect.

Changes in concentrations of some groups of pesticide residues during heat treatment are not significant. For example, the decrease in concentrations of synthetic pyrethroids during cooking, baking or frying of foods of plant origin is negligible. Similarly, the decrease of thiabendazole concentration during the baking of potatoes is also negligible.

Milling

The active ingredients of pesticide products used in the treatment of grains in the postharvest period are found mainly in the surface layers of the grains. The levels of residues present in bran are therefore high. Concentration factors of frequently used organophosphorus insecticides and synthetic pyrethroids thus often reach values in the range of 2–4. For products made from wholemeal flour, however, the risk of residues is higher than for products made from white flour (Table 12.63). The husking (de-hulling) process for removing the rice hull from the rough rice removes up to 90% of the pesticide residues.

12.6.3.2.2 Vegetable oils and animal fats

The treatment of oilseed plants during the growing period leaves only very low or undetectable pesticide residues, but the risk of contamination is significantly higher in the case of postharvest application of pesticides. In particular, relatively lipophilic

Table 12.63 Influence of wheat processing on pesticide content in cereal products.

Pesticide	Pesticide content in cereal products (%)[a]				Concentration factor
	Wholemeal flour	White flour	Whole grain bread	White bread	Bran/Wheat
Bioresmethrin[b]	5	64	43	80	4.0
Deltamethrin[c]	29	91	69	94	3.3
Diflubenzurone	31	83	22	66	2.2
Glyphosate	46	55	64	93	2.2
Permethrin	9	65	68	84	3.2

[a] Content in wheat = 100%.
[b] Bioresmethrin occurs in resmethrin and makes 35–40% of that insecticide.
[c] Unlike other pyrethroids, deltamethrin consists of one pure compound.

Table 12.64 Distribution and changes of selected pesticides in production of vegetable oils.

Pesticide	Raw material	Concentration factor raw oil/seeds	Loss of pesticide during deodorisation (%)
Dichlorvos	Soybeans	5.3	100
Etrimfos	Rapeseeds	2.7	98
Chlorpyrifos	Soybeans	4.1	100
Malathione	Soybeans	3.9	100
Parathione	Olives	4.5	-
Pirimifos-methyl	Peanuts	1.7	-
Permethrin	Sunflower seeds	0.7	17

pesticide residues pass to the crude oil during pressing or extraction. Concentration factors >1 were found primarily for the group of synthetic pyrethroids (such as cyhalothrin, cypermethrin, fenvalerate and permethrin) and some organophosphates (diazinone, dichlorvos, etrimfos, malathione, parathione, pirimifos-methyl and chlorpyrifos). Pesticide residues can be found especially in virgin oils, but are found only sporadically in refined oils (mostly traces of pyrethroids). A significant reduction of pesticide concentrations occurs during oil refining, especially during deodorising (Table 12.64).

12.6.3.2.3 Fruit juices

The amounts of pesticides passing from raw materials into fruit juices depend on the distribution of residues between the solid (skin or flesh) and liquid fruit portions. Moderately to highly lipophilic pesticides, such as parathion, captan, folpet or synthetic pyrethroids, usually pass to the raw juices only to a very limited extent. A further decrease of their contents may occur during operations such as clarification or ultrafiltration. The risk of infiltration of residues into the human food chain, however, exists when the solid waste with higher concentrations of pesticides is used as an animal feed.

12.6.3.2.4 Alcoholic beverages

The transition of residues of pesticides used to protect the grapes as far as the final product depends on their solubility in water and the rate of their degradation, particularly hydrolysis. For example, a total transfer of polar benomyl residues to final products can be expected. Conversely, a lower transition of frequently used fungicides such as folpet, captafol, propiconazole, triadimefon and vinclozolin from grapes to wine can be expected, mainly due to sorption of these compounds to the solid waste (Table 12.65). In beer, pesticide residues are found only exceptionally, due not only to dilution, but also to degradation during technological operations and sorption to yeast cells.

12.6.3.3 Residues in foods

The presence of pesticide residues in the human diet is mainly due to their targeted preharvest and postharvest applications. The source of contamination of agricultural crops by some more persistent compounds may be atmospheric transport from distant places. It is necessary to consider the possibility of translocation of residues from soil contaminated in previous growing seasons; various agrochemicals may also contain water from rivers and

Table 12.65 Changes in levels of selected pesticides in wine production (%).

Pesticide[a]	Must	Clarified must	Wine	Pesticide[a]	Must	Clarified must	Pesticide
Benomyl	0	0	0.8	Iprodion	45–70	60–80	70–78
Captafol	50	95	100	Metalaxyl	0	30–50	66
Folpet	50	95	100	Vinclozolin	59–88	80	89–93

[a]Pesticide content in grapes = 100%.

reservoirs. Foods of plant origin contain mainly residues of modern pesticides or their metabolites. Foods of animal origin may become a source of persistent organochlorine pesticides that accumulate in fat tissues. Residues of modern pesticides occur rather rarely and their presence can be attributed, for example, to contamination of feeds or to pesticides used in protecting animals. For illustration, Table 12.66 brings together data obtained from reports on pesticide monitoring published in various countries. Only pesticides found in more than 10% of cases during the investigation of the given commodity are summarised. In particular, pesticide residues are frequently found in cereals, where all of them are used in postharvest applications. Particularly

Table 12.66 Combination pesticide/commodity with a frequent incidence of positive residue findings (monitoring in different countries).

Pesticide	Commodity	Number of analysed samples	Samples with pesticide resudues (%)
Carbaryl	Barley	188	17.6
Carbaryl	Oat flakes	130	33.9
Carbaryl	Sorghum	156	96.8
Chlorpyrifos	Kiwi	127	20.5
Chlorpyrifos	Tomatoes	3613	27.8
Chlorpyrifos	Bell peppers	1428	27.7
Chlorthalonil	Celery leaves	375	65.6
Dicofol	Citrus fruits	1022	14.5
Dimethoate	Green peas	837	11.5
Dithiocarbamates	Apples	135	19.9
Dithiocarbamates	Grapes	285	17.9
Dithiocarbamates	Tomatoes	866	27.8
Dithiocarbamates	Cucumbers	613	11.9
Endosulfan	Spinach	183	21.9
Endosulfan	Melons	925	26.9
Endosulfan	Lettuce	2169	16.8
Endosulfan	Cucumbers	1659	19.8
Endosulfan	Green beans	574	11.7
Fenitrothione	Cereals	12 759	74.5
Permethrin	Celery leaves	422	15.4
Permethrin	Lettuce	2451	18.3
Permethrin	Spinach	183	12.6
Vinclozolin	Strawberries	509	48.1
Vinclozolin	Kiwi	126	43.7

frequent are findings of residues in fruits and vegetables with a waxy surface. Taking into account all available data (more than 200 combinations pesticide/commodity), the most commonly detected pesticides include acephate, benomyl/carbendazim, carbaryl, chlorothalonil, chlorpyrifos, cypermethrin, deltamethrin, dicofol, dimethoate, dithiocarbamates, endosulfan, fenitrothione, fenvalerate, malathione, parathione, permethrin and vinclozolin. Only a small proportion of residues exceed the values set by the national health limits. The upper limit of incidence of residues above the limit stated is 5% in most sources, but mutual comparability of data is very difficult for many reasons.

12.6.4 Health and toxicological assessment

Pesticides can be naturally toxic to non-target organisms, including humans, in particular if the effect of a substance is based on interference with similar processes, which take place in the human organism. In terms of the possibility of pesticide poisoning, the most discussed risks are associated with long-term exposure to residues obtained in the diet. Generally, the level of these risks, as well as other high-risk groups of food components, are derived not only from the dose received, but also from the mechanism of absorption, distribution, metabolism and excretion of pesticides from the body. A general methodology of pesticide toxicity classification was issued by the WHO.

The mechanism of toxic effects to mammals is accurately described for only a few groups of pesticides. As already mentioned, the toxicity of organophosphates and carbamates is due to inhibition of acetylcholinesterase, toxic effects of dinitrophenols and polychlorinated phenols lie in the inhibition of oxidative phosphorylation. The increased toxicity of certain pesticides may proceed *in vivo* by mechanisms that have been previously listed. This mainly concerns the formation of oxoanalogues of organophosphates, which are more potent acetylcholinesterase inhibitors than the parent thionophosphates and thiophosphates. Pesticides that accumulate in human fat (DDT, lindane and others), although not easily metabolised, may be mobilised under certain conditions (e.g. during starvation or breast feeding), which may lead to a significant increase in their concentration in blood serum with the manifestation of toxic effects.

None of the pesticides used today are classified by the IARC as a human carcinogen, yet some of them (particularly the classic pesticides DDT and toxaphene and modern pesticides amitrole and phenoxyalkanoic acids) are classified as potential human carcinogens. In recent years, in the context of assessing the exposure risks, the ability of certain pesticides to interfere with hormonal processes of vertebrates, including humans, and to cause so many side effects, is often discussed. Pesticides which show oestrogenic effects as xenooestrogens (anthropogenic oestrogens) include only some organochlorinated pesticides with high accumulation potential, such as toxaphene, dieldrin, heptachlor, DDT and derived compounds, especially DDE, but also modern pesticides represented by herbicides 2,4-D, atrazine and alachlor, fungicides benomyl and ethylenebisdithiocarbamates and insecticides carbaryl, dicofol, permethrin, parathione and others.

12.6.5 Mitigation

Legislative measures concerning the presence of pesticide residues in food are generally set at the EU level. Limits introduced in the past in national regulations were harmonised with the relevant EU limits. Maximum residue limits of pesticides (MRLs) in foods and crops and rules for their application are currently set at two different levels – global (Codex Alimentarius) and European, and in each case the limits for a large number of pesticide–commodity combinations are always given. Based on Regulation (EC) No. 396/2005 from September 2008, all responsibility for assessing the risk associated with the MRLs of pesticides was transferred to the European Food Safety Authority (EFSA), which is responsible for assessing the risks of new MRLs, but also for the periodic review of existing MRLs. Currently, EFSA coordinates revision of MRLs for more than 300 substances. In the case of previously used persistent pesticides that are still found in foods due to contamination of the ecosystem, the so-called Extraneous Residue Limits (ERLs) are set. Unlike in other groups of contaminants, inputs of which into the human food chain are difficult to completely eliminate, in the case of pesticides alternative ways to protect crops from harmful agents to minimise pesticide residues are increasingly being sought. In particular, emphasis is put on the integrated protection incorporating complementary practices, including breeding of resistant plants and the use of biological pesticides.

For foods listed in Annex I to Regulation (EC) No. 396/2005 (315 foods, including fruits, vegetables, cereals, spices and foods of animal origin), if the relevant MRL value for a particular pesticide is not declared in Annexes II (list of MRL values) or III (temporary EU limits), then the default limit is 0.01 mg/kg. This of course does not apply to pesticides referred to in Annex IV (52 pesticides for which MRLs are not declared, referring to the low risk) or Annex V (pesticides with other limits than 0.01 mg/kg). Annexes to the Regulation (EC) No. 396/2005 are gradually amended by other extensive regulations. Owing to the vast number of MRLs given by combinations of several hundred pesticides with many types of food, and due also to the need for constant updating of the pronounced values, the legislation concerning pesticides is very confusing and difficult to use in practice.[9] Carcinogenic and mutagenic substances, or substances acting as endocrine disrupters, are banned. However, they may obtain exemption if there is no substitute and they are proved to be necessary for pest control. Immunotoxic and neurotoxic pesticides are included in the list of candidates that will be replaced with safer alternatives where such alternatives exist.

12.7 Veterinary drugs

The production of high quality and hygienically acceptable foods of animal origin in agriculture implies ensuring the overall health and welfare of livestock through good nutrition and reducing

[9] Current MRLs can be found at http://ec.europa.eu/sanco_pesticides/public/index.cfm.

stress. Livestock production is therefore not possible without the use of veterinary drugs. Veterinary drugs (pharmaceuticals) are pharmacologically and biologically active chemicals used for the treatment, prevention and diagnosis of diseases in animals. With ever-increasing demands on productivity, growth in livestock production is manifested by the high concentration of animals in factory farms and their frequent geographic relocation, which promotes the spread of infectious diseases. As a result, the use of veterinary drugs in animal production has greatly increased over the last few decades. In pursuit of high productivity, inadequate sanitary conditions were in many cases replaced by increased use of pharmaceuticals, with the aim of preventing deterioration of the health of farm animals. An extreme way of increasing productivity can be the illegal use of pharmacologically active compounds as growth promoters, such as anabolic steroids, thyreostatics and antibiotics in low doses.

12.7.1 Classification, structure and properties

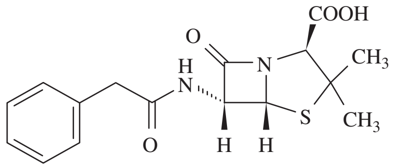
The veterinary products for a variety of animals, including pets, livestock and numerous exotic species, comprise several hundreds of pharmaceutical, biological, diagnostic, feed medications and parasiticide products. The most widely used groups of veterinary drugs include:

- antimicrobial substances
- antiparasitic agents.

Also often used as veterinary drugs are:

- anti-inflammatory agents called **antiflogistics**
- drugs acting on the nervous system, which include anaesthetising agents or **anesthetics** and sedative agents or **tranquilisors**
- drugs acting on the kidney, known as **diuretics**
- preparations acting on the digestive tract, such as against diarrhoea that are called **antidiarrhoics**
- hormones
- vitamins
- trace mineral elements and others.

In the European Union, approximately 700 pharmacologically or biologically active chemical compounds are currently either lodged for registration or registered for use in the Member States. Examples of the most important groups of pharmacologically active substances used in veterinary medicine are given in Tables 12.67 and 12.68. The structures of selected pharmaceuticals are illustrated by formulae **12-201** to **12-230**.



12-201, benzylpenicillin

Table 12.67 List of frequently used veterinary antimicrobial drugs.

Pharmaceuticals	Name	Pharmaceuticals	Name
Antibiotics		Macrolide	Tylosin
β-Lactam			Spiramycin (selectomycin)[a]
Penicillins	Benzylpenicillin (penicillin G)		Erythromycin[b]
	Ampicillin	Chloramphenicol group	Chloramphenicol
	Amoxicillin		Thiamphenicol
	Cloxacillin	Lincosamides	Lincomycin
Cephalosporins	Ceftiofur		
	Cephaloridine	***Sulfonamides***	Sulfadiazine
Tetracycline	Tetracyclin		Sulfadimidine
	Chlortetracycline		Sulfadoxine
	Oxytetracycline		Sulfadimethoxine (dinosol)
Aminoglycoside	Dihydrostreptomycin		Trimethoprim
	Gentamycin		
	Kanamycin[c]	***Quinolones***	Enrofloxacin

[a] Contains three components: spiramycins I, II and III.

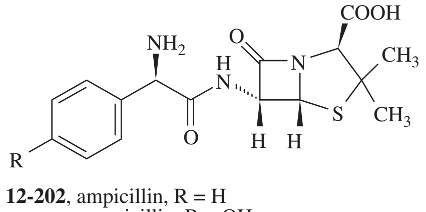
[b] Erythromycin A is the main metabolite, other components are erythromycin B (without an OH group in C-12 position of aglycone called erythronolide) and erythromycin C, which contains instead of cladinose (2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-L-*ribo*-hexopyranose also known as 3-*O*-methylmycarose) saccharide known as mycarose.

[c] Pharmaceuticals contain 95% of kanamycin A and 5% of kanamycin B; kanamycin C is produced from kanamycin B.

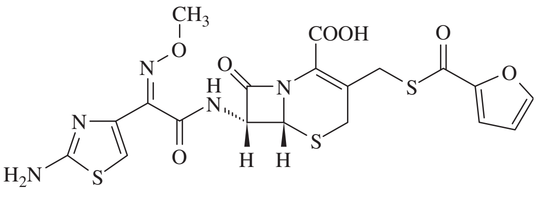
Table 12.68 Other important groups of veterinary drugs.

Pharmaceuticals	Name[a]
Antiparasitic agents[b]	
Coccidiostats[c]	Monensin, dicarbazim, narazin
Anthelmintics[d]	
Benzimidazoles[e]	Thiabendazole, albendazole
Probenzimidazoles[e]	Netobimin, febantel
Macrolide endectocides[f]	Ivermectin,[l] doramectin
Pyrethroids[g]	Permethrin
Organophosphates[g]	Foxim
Other agents	
Non-steroid anti-inflammatory agents (antiflogistics)	Carprofen, ketoprofen
Analgetics, antipyretics[h]	Salicylic acid
Substances effecting the autonomic nervous system[i]	Clenbuterol
Substances effecting the central nervous system	Pentobarbital Amphetamine[m]
Sedatives[j]	
Analeptics[k]	

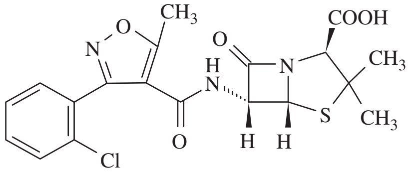
[a]Thiabendazole and permethrin structures (see Section 12.6.2.1.2), salicylic acid structure (see Section 8.2.6.1.6).
[b]Drugs used against external (ectoparasitica) and internal (endoparasitica) parasites.
[c]Drugs used primarily to protect chickens against coccidiosis (enteritis or cachexia).
[d]Drugs used in the treatment of helmintoses, infections caused by lower parasitic worms.
[e]Endoparasitica.
[f]Endoparasitica and ectoparasitica.
[g]Ectoparasitica (insecticides).
[h]Analgesics are drugs that decrease or inhibit the perception of pain of various origins, antipyretics reduce abnormally elevated body temperature, while they do not affect the normal body temperature (often also having an analgesic effect).
[i]Bronchodilator effect.
[j]Drugs causing a decrease of increased excitability of the central nervous system.
[k]Drugs with stimulating effect on the central nervous system acting on circulatory and respiratory centra in the medulla.
[l]Contains about 80% of dihydroavermectin B_{Ia} (22,23-dihydroavermectin) and 20% of dihydroavermectin B_{Ib}.
[m]Mixture of enantiomers.



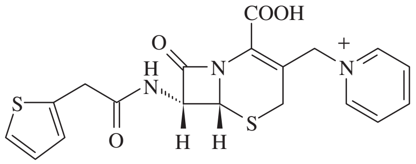
12-202, ampicillin, R = H
amoxicillin, R = OH



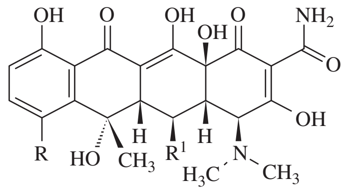
12-204, ceftiofur



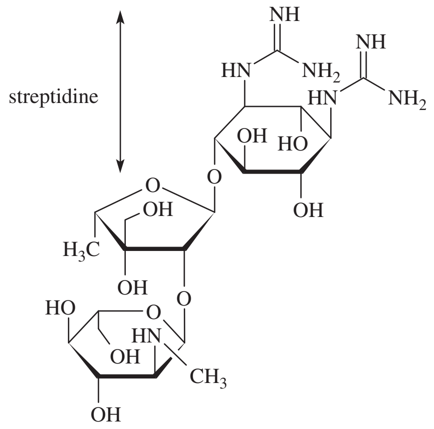
12-203, cloxacillin



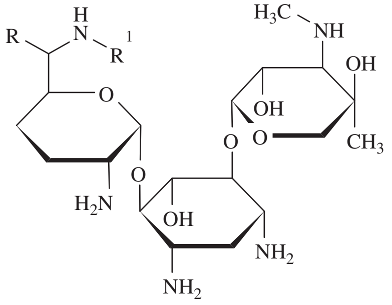
12-205, cephaloridine



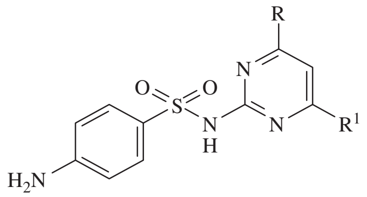
12-206, tetracycline, $R = R^1 = H$
chlortetracycline, $R = Cl$, $R^1 = H$
oxytetracycline, $R = H$, $R^1 = OH$



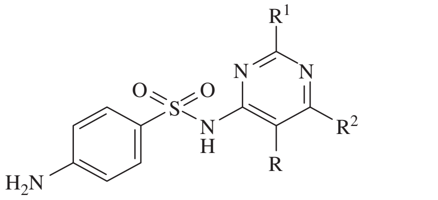
12-207, dihydrostreptomycin



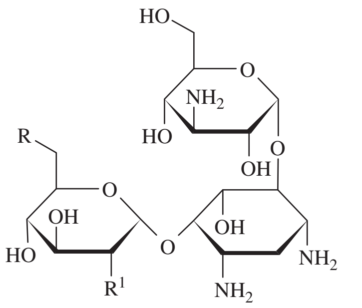
12-208, gentamicin C_1, $R = R^1 = CH_3$
gentamicin C_2, $R = CH_3$, $R^1 = H$
gentamicin C_3, $R = R^1 = H$



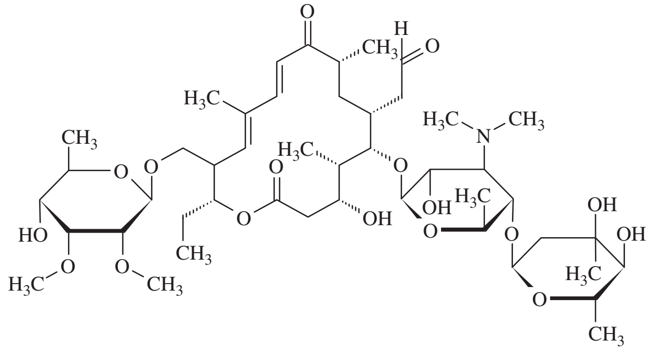
12-209, sulfadiazine, R = R1 = H
sulfadimidine, R = R1 = CH_3



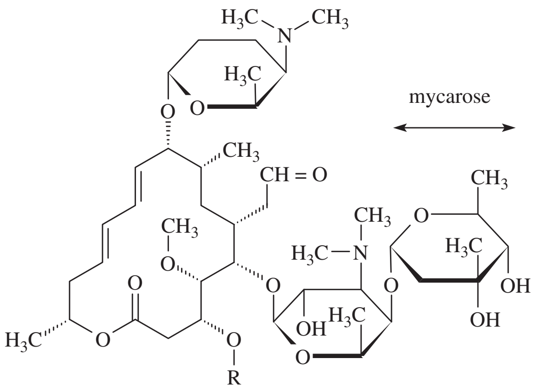
12-210, sulfadoxine, $R = R^1 = OCH_3$, $R^2 = H$
sulfadimethoxine, $R = H$, $R^1 = R^2 = OCH_3$



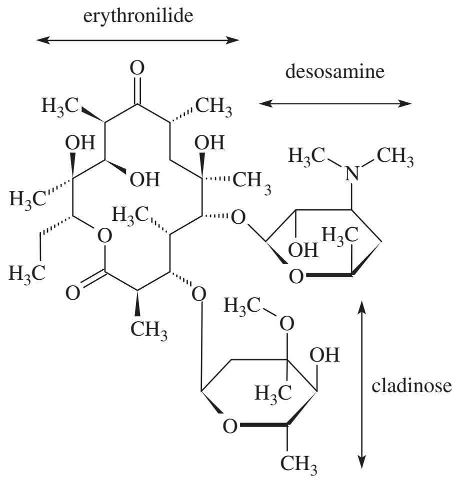
12-211, kanamycin A, $R = NH_2$, $R^1 = OH$
kanamycin B, $R = NH_2$, $R^1 = NH_2$
kanamycin C, $R = OH$, $R^1 = NH_2$



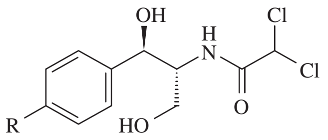
12-212, tylosin



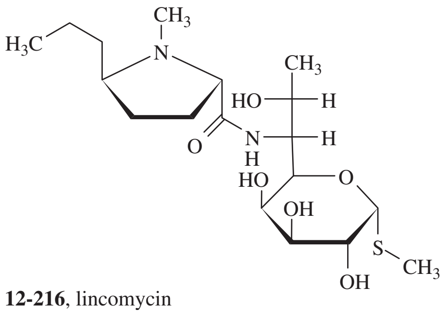
12-213, spiramycin I, R = H
spiramycin II, $R = C(=O)CH_3$
spiramycin III, $R = C(=O)CH_2CH_3$



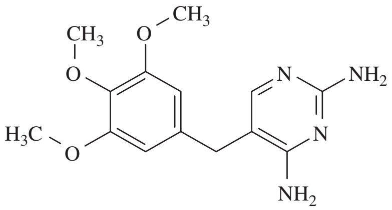
12-214, erythromycin A



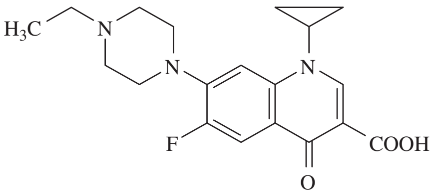
12-215, chloramphenicol, R = NO_2
thiamphenicol, R = SO_2CH_3



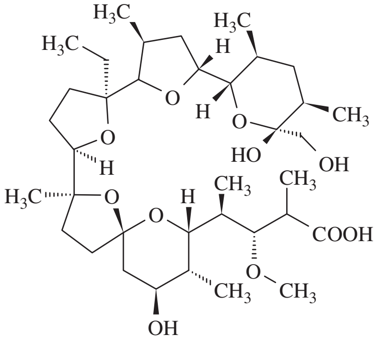
12-216, lincomycin



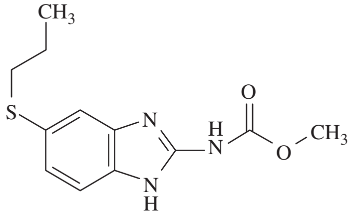
12-217, trimethoprim



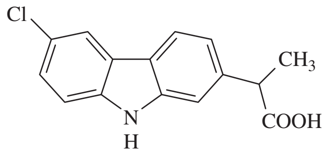
12-218, enrofloxacin



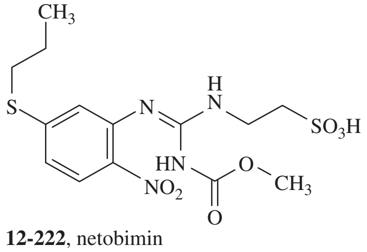
12-219, monensin



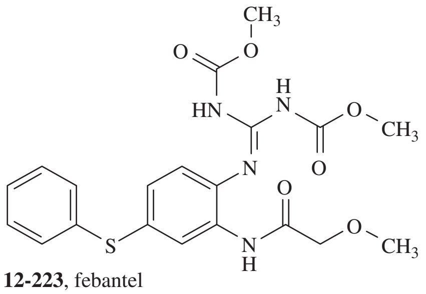
12-220, albendazol



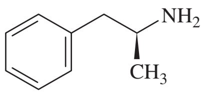
12-221, carprofen



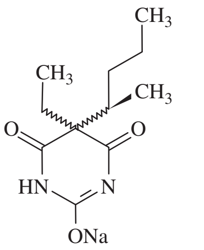
12-222, netobimin



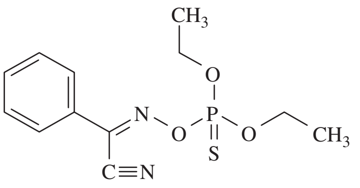
12-223, febantel



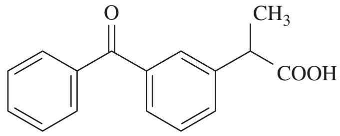
12-224, (*S*)-amphetamine



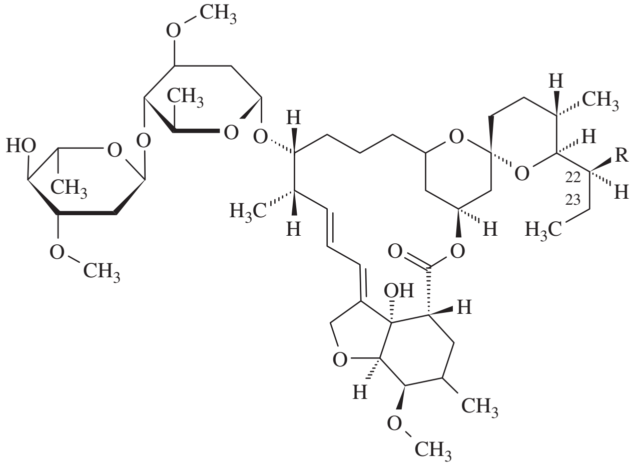
12-225, (+)-(*R*)-pentobarbital (sodium salt)



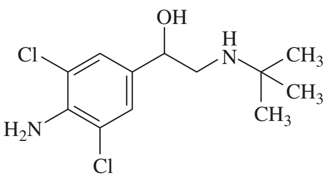
12-226, foxim



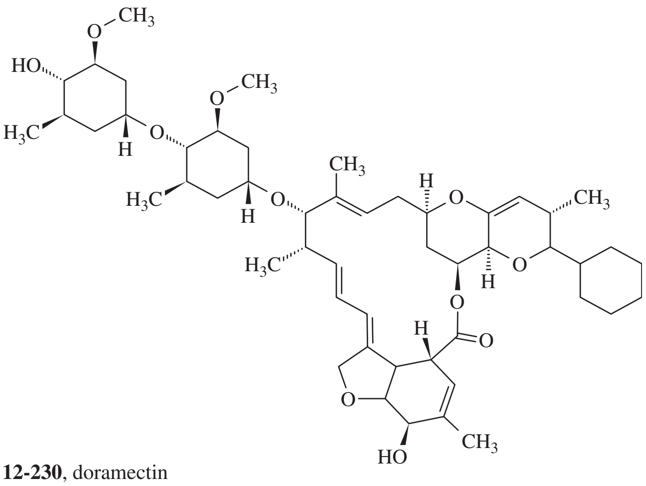
12-227, ketoprofen



12-228, dihydroavermectin B_{Ia}, R = CH_2CH_3
dihydroavermectin B_{Ib}, R = CH_3



12-229, clenbuterol



12-230, doramectin

12.7.2 Occurrence in foods

The supply of veterinary drugs into the body of livestock necessarily leads to the occurrence of residues in the muscle, various organs, milk and eggs, so trace amounts of veterinary drugs may be correspondingly found in meat, milk, meat, dairy and other products.

In terms of food quality, veterinary drugs are considered extraneous, contaminating substances whose presence is undesirable, although often inevitable. In this context, these substances are classified in the same group as environmental contaminants, such as mycotoxins, pesticides, polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs). Like other types of contaminants, residues of veterinary drugs in foods present a potential health risk to the consumer. Therefore, the incidence and levels of veterinary drug residues in tissues have to be effectively and consistently controlled.

12.7.2.1 Residues in tissues

The levels of veterinary drug residues are subject to pharmacokinetic parameters: absorption, tissue distribution, metabolism and excretion. The degree of absorption depends on the physico-chemical properties of the compounds and the method of administration, which may, for example, be:

- **peroral** (through the gastrointestinal tract: such as in capsules, food or drinking water)
- injected, **intramuscular** (within a muscle) or **subcutaneously** (under the skin)
- **dermal** (on skin surface).

Generally speaking, the absorption from the gastrointestinal tract depends on the solubility of the drug in water (degree of ionisation at a given pH) and lipophilicity, which determines the transition across biological membranes. Lipophilic compounds, such as certain antiparasitic drugs (organophosphates and pyrethroids), exhibit a relatively high degree of absorption after application to the skin. On the other hand, the injection preparations in the form of an oil suspension can lead to up to several months of persistence of residues at the injection site, with the risk of occurrence of extreme levels of residues in food (particularly in meat) prepared from the given tissue.

Accumulation of residues in the liver and kidneys generally exceeds levels in muscle. Some compounds, such as benzimidazoles, show the highest concentration in the liver, while the majority of antibiotics are located in the highest amounts in the kidney. Lipophilic chemical compounds, such as some organophosphates, accumulate in adipose tissue.

The most important factors affecting the transfer of veterinary drugs from the blood plasma into milk (mammary gland cells) are:

- degree of ionisation

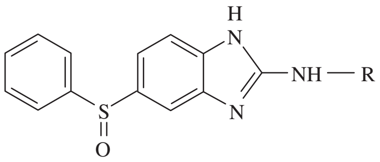
- lipophilicity
- binding to proteins
- relative molecular weight.

At the lower pH of milk, the ratio between milk and plasma for weak organic bases (such as antibiotics erythromycin and tylosin) is >1, while for organic acids (benzylpenicillin, sulfadimethoxin) it is ≤1. For example, during mammary gland tissue inflammation (mastitis), the pH of milk increases and this ratio of the organic bases decreases. Specific examples are given in Table 12.69.

12.7.2.2 Metabolism and excretion

The elimination of most veterinary drugs from the body depends on the metabolic rate and mechanism of the excretion process. Examples of differences in the rate of elimination of veterinary drugs from the body of selected animal species are presented in Table 12.70. Metabolism proceeds in two phases. The initial stage involves oxidation, reduction and hydrolysis, while the second phase leads to conjugation with endogenous components, for example with D-glucuronic acid and glutathione, or the drug is acetylated or otherwise transformed. In most cases, the metabolic process yields water-soluble, easily eliminated products that lack pharmacological activity. A summary of the most common biotransformation processes is given in Table 12.71.

In some cases, however, one or more metabolites exhibit similar biological effects. An example is oxfendazole (**12-231**) in the tissues, which is in equilibrium with the corresponding sulfide (fenbendazole), which shows an anthelmintic effect, and with some other metabolites (such as the corresponding sulfone), which already lack pharmacological activity. A related compound is febantel (**12-223**) from which oxfendazole is produced *in vivo*.



12-231, oxfendazole, R = $COOCH_3$
oxfendazole amine, R = H

The major excretory organs are the kidneys, but veterinary drug metabolites can also be excreted to the bile and milk. Renal excretion is the primary elimination for drugs that are ionised at physiological pH, and compounds with low lipophilicity. Drugs excreted unchanged include many antibiotics, such as penicillin, cephalosporins, and aminoglycosides, oxytetracycline and most diuretics. The process of renal excretion of drugs is very complex, therefore in addition to the parent compound, pharmacologically

Table 12.69 Transition of antimicrobial substances from the blood plasma into milk.

Veterinary drug	pK_a	pH of milk	Ration of concentrations Milk ultrafiltrate/ plasma ultrafiltrate	
			Theoretical	Experimental
Acids				
Benzylpenicillin	2.7	6.8	0.25	0.13–0.26
Ampicillin	2.7; 7.2	6.8	0.26	0.24–0.30
Cloxacillin	2.7	6.8	0.25	0.25–0.30
Cefaloridine	3.4	6.8	0.25	0.24–0.28
Sulfadimidine	7.4	6.6	0.58	0.59
Sulfadimethoxine	6.1	6.1	0.20	0.23
Bases				
Kanamycin	(7.8)	6.8	3.1	0.60–0.80
Tylosin	7.1	6.8	2.0	3.5
Erythromycin	8.8	6.8	3.9	8.7
Lincomycin	7.6	6.8	2.83	2.50–3.60
Trimethoprim	7.6	6.5–6.8	2.8–5.3	2.90–4.90
Amphoteric substances				
Oxytetracycline	-	6.5	-	0.75

Table 12.70 Half-life of elimination (hours) of veterinary drugs from the body of livestock.

Veterinary drug	Cattle	Horse	Pig
Hepatic metabolism			
Chloramphenicol	4.2	0.9	1.3
Salicylic acid	0.8	1.0	5.9
Pentobarbital	0.8	1.5	-
Amfetamine	0.6	1.4	1.1
Hepatic metabolism and renal excretion			
Sulfadoxine	11.7	14.0	8.2
Sulfadimethoxine	12.5	11.3	15.5
Trimethoprim	1.5	3.2	2.3
Renal excretion			
Benzylpenicillin	0.7	0.9	-
Ampicillin	1.2	1.6	-
Oxytetracycline	9.1	10.5	-
Kanamycin	1.9	1.5	-

Table 12.71 Various ways of biotransformation of veterinary drugs.

Functional group/skeleton	Biotransformation
Aromatic ring	Hydroxylation
Hydroxyl group	
Alcohols	Side chain oxidation, conjugation with glucuronic acid and sulfate (to a lesser extent)
Phenols	Ring hydroxylation, conjugation with glucuronic acid and sulfate, methylation
Carboxylic group	Conjugation, side chain hydroxylation
Alifatic and aromatic substances	Conjugation with glucuronic acid and glycine
Primary amines	Deamination
Alifatic	Side chain hydroxylation, acetylation
Aromatic	Conjugation with glucuronic acid and sulfate, methylation
Sulfhydryl group	Conjugation with glucuronic acid, methylation, oxidation
Ester and amide bonds	Hydrolysis

active metabolites must always be analysed. Individual maximum residue limits (MRLs) are set according to the occurrence of residues in the body for the meat and various edible organs, such as the liver and kidneys.

12.7.2.3 Effect of heat treatment

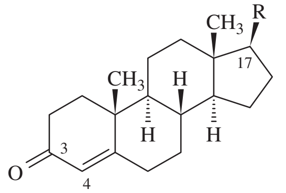
Until recently, there were surprisingly few studies dealing with the impact of processed raw materials of animal origin on residues in consumed foods. Knowledge of this area is still too limited to allow any generally valid conclusions. For example, cooking and baking meat containing residues of the antiparasitic drug ivermectin leads to reduced levels of this drug, on average down to 50%. Similarly, oxytetracycline residue levels decrease by 35–94% depending on temperature and type of heat treatment. On the other hand, under standard cooking or baking procedures no changes were found in the concentration of residues of clenbuterol, which is sometimes used illegally as a growth stimulator. Frying of beef liver containing the antiparasitic drug oxfendazole leads to changes in the relative ratio of oxfendazole residues and the main metabolites, such as oxfendazole sulfone, fenbendazole and some others. Oxfendazole amine is produced as a product of hydrolysis of oxfendazole.

12.7.3 Health and toxicological evaluation

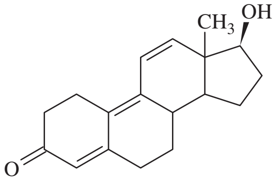
In recent years increasing attention has been devoted at both the national level and by international organisations to the risk assessment of veterinary drugs occurring in foods, and to the implementation of effective measures to reduce this risk.

12.7.3.1 Hormones as growth promoters

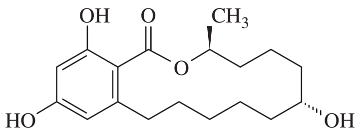
The use of hormones as growth promoters is banned in the EU. Other states, namely the United States, Australia and Argentina, allow the controlled use of hormonal preparations. The drugs used include natural steroid hormones which have an anabolic effect, such as oestradiol, (17β)-oestra-1,3,5(10)-trien-3,17-diol (see **10-93**), testosterone, (17β)-hydroxyandrost-4-en-3-one, or progesterone, pregn-4-en-3,20-dione (**12-232**). Another group consists of synthetic hormones with corresponding effects, such as trenbolone (17β)-17-hydroxyoestra-4,9,11-triene-3-one (**12-233**) or non-steroidal substances having an oestrogenic effect, such as zearalenol (α-zearalenol, the product of reduction of mycotoxin zearalenone, Figure 12.43) and α-zearalanol (**12-234**), with



12-232, testosterone, R = OH
progesterone, R = $COCH_3$



12-233, trenbolone

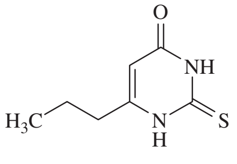


12-234, α-zearalanol

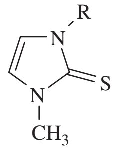
a reduced double bond, which is also called zeranol in veterinary terminology.

The opinions of professionals as to the potential risks from hormone residues are not uniform. Their potential carcinogenic effects in humans (particularly in reproductive organs and mammary glands) are the areas predominantly discussed. Altogether it is considered that the residual amounts of used hormones are low and are within the normal, physiological values of endogenous hormones, so that there is no significant risk to the consumer health. Their ban in European countries is mainly based on the negative attitudes of the general public to this type of increased production. In this context, other legitimate factors which are unacceptable from an ethical point of view are also discussed, such as the burden upon the animal by excessive growth stimulation or excessive production of milk or eggs.

Similar effects to those for hormones are seen in antithyroid substances (strumigens), which inhibit the synthesis of thyroxine in the thyroid gland. Heterocyclic compounds containing thiourea residues in their molecules, for example thiouracils and mercaptoimidazoles, are very potent strumigens. Examples of the main antithyroid drugs include propylthiouracil (6-propyl-2-sulfanylpyrimidin-4-one, **12-235**), which was in the past used in human medicine. In 2009, the U.S. Food and Drug Administartion (FDA) published an alert notifying healthcare professionals of the risk of serious liver injury, including liver failure and death, related to the use of propylthiouracil. As a result, propylthiouracil is no longer recommended for non-pregnant adults or children. Examples of other antithyroid agents are methimazole (1-methyl-3*H*-imidazole-2-thione, **12-236**) carbimazole (ethyl 3-methyl-2-sulfanylideneimidazole-1-carboxylate, **12-236**), which acts as a pro-drug, because after absorption it is converted into the active form, methimazole.



12-235, propylthiouracil



12-236, methimazole, R = H
carbimazole, R = $COOCH_2CH_3$

12.7.3.2 Risk of bacterial resistance

There is also evidence that the excessive and indiscriminate use of drugs in veterinary medicine leads to some undesirable effects. Discussions take place at the international level, especially in the case of antibiotics, the excessive use of which may result in resistance of pathogenic microorganisms in the tissues of treated animals, which reduces the effectiveness of the drugs. A similar phenomenon is known in human medicine, where effective antibiotics are very difficult to find for certain infections. A very important issue is the risk of transmission of resistance in livestock pathogens from one strain to another. Even more serious is the possibility of infection of food consumers with resistant strains of pathogenic microorganisms or induced cross-resistance to antibiotics used in human medicine only. A typical example is the finding from Denmark in 1995, when the preventive use of the antibiotic avoparcin (used as a feed additive to promote growth in chickens and pigs) led to the emergence of the resistant enterococci *Enterococcus faecium*. Based on the structural similarity, this strain, which was highly pathogenic to humans, also showed cross-resistance to vancomycin, which is only used against pathogenic enterococci in human medicine. *E. faecium* resistant to vancomycin was also isolated from raw chicken and pork from the retail network. Based on the above information, the use of avoparcin was banned as an additive in animal feed. Under pressure (especially in Scandinavian countries), in 1999 the EU member states also prohibited the use of other antibiotics (bacitracin, tylosin, virginiamycin and spiramycin) as growth stimulators in animal feed.

12.7.3.3 Mitigation

The actual production, distribution and testing of veterinary drugs (including medicated feed) are summarised in The Rules Governing Medicinal Products in the European Union (Eudralex).[10] The approval process for a new veterinary drug in the EU includes, according to Regulation (EC) No. 726/2004, the evaluation of its therapeutic efficacy, side effects in animals, environmental impact and ensured good manufacturing practice. Documentation provided by the manufacturers (pharmaceutical companies) must include information about the structure of substances, the recommended route of administration and dosage, pharmacokinetics (absorption from the gastrointestinal tract, distribution and accumulation in tissues, metabolism and excretion in farm and laboratory animals), acute (short-term) toxicity (single dose), subchronic and chronic toxicity (repeated daily dosing of laboratory animals for ≥90 days in two species, such as mouse and dog), embryotoxicity (disorders of foetus and fertility development), mutagenicity (such as gene mutations, chromosomal disordersand DNA reparation), carcinogenicity (ability to cause tumour growth through daily, lifelong use by laboratory animals), specific effects as needed (such as neurotoxicity and allergies), the influence of

[10] Details can be find at http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/eudralex_en.htm.

substances with antimicrobial properties to the normal composition of intestinal microflora and cultural microorganisms used in technological processes (fermentation), adverse reactions observed in treated animals, and the side effects of eventual use in humans.

For some veterinary drugs, as in the case of pesticides, a withdrawal time is set, defined as the time required after administration of a drug to assure that drug residues in the marketable product (e.g. meat, milk and eggs) are below a determined maximum residue limit (MRL). The reasons for the introduction of withdrawal times are not only toxicological (protection against potentially harmful effects of residues on the consumer's health), but also technological (protection of fermentation processes against residues of antimicrobial agents) and ethical (to prevent involuntary exposure of consumers to therapeutic doses of drugs in food). The length of withdrawal periods, determined for the individual form of drug (including those taken by injection, oral intake and others), animal species and raw materials (meat, milk and eggs) can vary from several days to several weeks, and in exceptional cases to several months.

Maximum residue limits of veterinary medicinal products in foods of animal origin established according to Council regulation (EEC) No. 2377/90 represent internationally generally accepted thresholds, which indicate the amounts of drug residues that may occur in foods of animal origin. With regard to the character of veterinary drugs, MRLs are classified into four groups:

- group I, where MRLs are set
- group II, where MRLs are not set (with respect to the identified harmlessness for human health)
- group III, where MRLs are set only temporarily in the European Union
- group IV, where drugs dangerous for human health are listed, which can not be used on animals intended for food production.

Group I includes numerous chemotherapeutics and antibiotics, non-steroidal anti-inflammatory drugs, corticosteroids, antiprotozoa agents, endo- and ectoparasitica and drugs affecting the nervous and reproductive systems. Examples of MRL values (reflecting the diversity of species and distribution of residues between various tissues and organs of animals) are listed in Table 12.72. Group II contains mainly inorganic substances, such as chlorides, sulfates, phosphates, gluconates, carbonates, vitamins and other organic compounds (such as acetylsalicylate and alcohols), herbs, tinctures, homeopathics and other substances generally recognised as safe for the intended use. The fact that a substance is included in group II does not mean that its use is generally permitted. The legislation typically clearly defines the target organism and method of use. Great attention of control systems at the national and European level is focused on agents assigned in group IV, which include, for example, chloroform, chloramphenicol and chlorpromazine, which must not be present (detected) in animal products at all. In accordance with European legislation, prohibited substances are those with thyreostatic, oestrogenic, androgenic or gestagenic activities and some sympathomimetic agents (that stimulate the heart through activation of β-adrenoceptors; β-adrenoceptor agonists, also known as β-agonists, bind to β-receptors on cardiac and smooth muscle tissues). Legislation also includes 17β-oestradiol, stilbenes and all its derivatives. To allow the free trade of animal products within the EU, each member state must carry out official control and monitoring of residues of pharmaceuticals and biologically active substances.

Table 12.72 Examples of maximum residue limits for benzylpenicillin and ivermectin.

Animals	MRL (mg/kg)	Raw material
Benzylpenicillin		
All species	0.050	Meat, liver, kidneys, fat
	0.004	Milk
Ivermectin (dihydroavermectin B_{Ia})		
Cattle	0.100	Liver
	0.040	Fat
Sheep	0.020	Fat
Horse	0.015	Liver

12.8 Contaminants from packaging materials

Packaging has become an indispensible element in the food manufacturing process. Packaging maintains the benefits of food processing after the process is complete, makes food more convenient, gives the food greater safety assurance from microorganisms, biological and chemical changes, and enables food to travel safely for long distances from their point of origin and still be wholesome at the time of consumption.

12.8.1 Occurrence and main sources

Contamination of food by components of packaging materials is one of the most serious health problems relating to food packaging. In almost all cases, when the food is in direct contact with the packaging material, a reciprocal mass transfer proceeds between the food and the food packaging components, even when relatively stable packaging materials are used, such as glass. In order to maintain food quality, inert materials of high quality should be used for food packaging. Food packaging is therefore more expensive to produce than the packaging of the majority of other goods. The most important and commonly used packaging materials, which are in direct contact with the packaged food and can thus significantly affect its quality, are:

- metals

- glass and ceramics
- paper
- wood
- plastics.

12.8.1.1 Metals

Metals are the most versatile packaging materials as they offer a combination of excellent physical protection and barrier properties, decorative potential and recyclability. Tin, steel and aluminium have long histories of successful use in storing food. The basic problem of metals in food packaging materials is metal corrosion, which occurs due to the action of food (mainly acidic food) on the metal container, which leads to partial dissolution of the package and results in higher metal content in the food. The maximum permissible levels of some metals in foods are given in Chapter 6. The mechanisms of corrosion are basically twofold. Chemical and electrochemical corrosions can be distinguished. The possible ways to prevent corrosion are very diverse and widely used (such as tinning and passivation of the package, and use of lacquered packages).

Just under one third of the world's total tin production goes into the manufacture of tinplate, a sheet or strip of low-carbon steel (blackplate) coated with a thin layer of pure tin. Tinplate has been used for preserving food for well over a hundred years as it is an excellent barrier to gases, water vapour, light and odours, and can be heat-treated, sealed hermetically and easily recycled. About 25 000 million food cans are produced and filled in Europe *per annum*, about 20% of these having plain internal (unlacquered) tin-coated steel bodies. Plain internal tinplate cans may be used, for example, for tomatoes and other tomato-based products, white fruits and some vegetables (such as asparagus and mushrooms). Although tin provides steel with some corrosion resistance, tinplate containers are often lacquered to provide an inert barrier between the metal and the food product. Food grade lacquers are based on natural resins and also on a number of synthetic lacquers, such as phenol-formaldehyde resins, epoxy and vinyl resins, polyesters and others. Sometimes, the lacquers used to protect the internal surfaces of metal packaging may be sources of food contaminants. Their effect on food contamination is analogous to the effect of polymeric packaging materials.

Tin-free steel used for food and beverage cans is chromium or chrome oxide coated steel that was developed to meet economic requirements and surpass tinplate in paintability and paint adhesion. It requires a coating of organic material to provide complete corrosion resistance.

Aluminium's natural resistance to corrosion is advantageous for its role in packaging as, unlike iron, aluminium oxide forms a protective and not a destructive layer. Aluminium also provides an excellent barrier to moisture, air, odours, light and microorganisms. One of the most common end uses for more than 20% of the aluminium manufactured is in packaging, including drinks cans, foil wrappings and bottle tops. Lamination of packaging involves the binding of aluminium foil to paper or plastic film to improve barrier properties. As well as recent health worries linking aluminium to Alzheimer's disease, the main disadvantages of aluminium are its high cost compared with other metals (e.g. steel) and its inability to be welded.

12.8.1.2 Glass

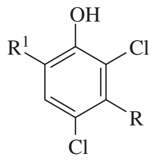
Glass has an extremely long history in food packaging, which is dates back to approximately 3000 BC. Glass is impermeable to gases and vapours, so it maintains product freshness for a long period of time without impairing its sensory properties and is reusable and recyclable. Glass is much more resistant to corrosion than most materials, so much so that it is easy to think of it as corrosion-proof. Nevertheless, glass may frequently be attacked by acidic solutions that remove the alkali from the glass surface and wash out ions of sodium, calcium and other alkali metals and alkaline earth metals with the formation of alkali silicates. These salts, if not removed by washing, can get into the food product. Water corrosion acts at a much slower rate than corrosion by acids, but may become important at high temperatures, especially during longer periods of rain or in case of condensation in glass containers which have been improperly stored before filling. In coloured glass, metal ions used for colouring (such as iron, manganese, chromium and some other metals) can be washed out, which may result in increased concentrations of these ions in packaged products.

12.8.1.3 Paper

The use of paper for food packaging dates back to the 17th century. Paper and paperboard are commonly used in corrugated boxes, milk cartons, bags and sacks and wrapping paper. Plain paper has poor barrier properties and poor resistance to moisture, and therefore is not usually suitable for direct contact with food of higher water activity or to protect food for long periods. When used in direct contact with food, paper is prepared from cellulose pulp by modified technological processes and it is often coated, laminated or impregnated with waxes, resins or lacquers to improve its functional and protective properties. Such products include papers treated with sulfate (kraft paper used to package, for example, flour and salt), sulfite (sulfite paper used, for example, to package confectionery), grease-proof paper (prepared by extended cellulose hydration), and parchment paper (prepared from sulfuric acid treated pulp). Paper laminates (paper coated, for example, with polyethylene or aluminium foil) are based on kraft and sulfite papers.

When in contact with food raw materials and foods under low moisture conditions, the migration of some paper components (such as fillers, binders, precipitation, fixation and retention agents, drainage accelerators, dispersants, flotation and antifoaming agents, preservatives, lubricants, optical brighteners, plasticisers and others) is possible. Possible contamination of food by paper laminates depends on the properties of these materials and is similar to the contamination caused by polymeric packaging materials. A special group are volatile substances, which may

be present in the paper and can pass into the packaged food. Such substances may include various chlorinated phenols (2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorfenol and other substances, **12-237**), which arise, for example, in bleached papers and cork stoppers, and carbon disulfide that may be found in kraft paper. Paper made from recycled paper, which is still only rarely used in contact with foods, may contain polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). Benzophenone (diphenyl ketone) may be present in cartonboard food-packaging materials as a residue from UV-cured inks and lacquers used to print on the packaging. It may also be present if the cartonboard is made from recycled fibres recovered from printed materials. The main odour-active compounds of cardboard include vanillin, (*E*)-non-2-enal, (R/S)-γ-nonalactone, 2-methoxyphenol, (R/S)-δ-decalactone, *p*-anisaldehyde and 3-propylphenol. Paper exposed to higher temperatures may contain degradation products of cellulose, such as 5-hydroxymethyl-2-furancarbaldehyde, which may adversely affect the flavour of packaged foods.



12-237, 2,4-dichlorophenol, R = H, R^1 = H
2,4,6-trichlorophenol, R = H, R^1 = Cl
2,3,4,6-tetrachlorophenol, R = Cl, R^1 = Cl

12.8.1.4 Wood

Wood packaging is generally unsuitable for direct contact with food as the wood components, in particular resins and tannins, are easily released to food products by leaching or volatilisation. Before their first use, packaging and containers intended for direct contact with food have to be treated with water and solutions of sodium carbonate or sulfites, or the inner surface has to be covered with a protective layer of, for example, polymer-based materials. Possibilities of food contamination are then dependent on the quality of the protective layer and the type of plastics. An exception is the storage of spirits and wines in oak barrels, where the extraction of wood components to the product is desirable.

12.8.1.5 Plastics

The production of plastic materials on an industrial scale began in the 1940s and 1950s, and world production reached 270 million tons in 2011 (57 million tons of which was in Europe). In 2011, food packaging applications used 39.4% of the worldwide plastics production. These materials included a range of organic polymers:

- **thermoplastics**, which soften upon exposure to heat and return to their original condition at room temperature;
- **thermosets** that solidify or set irreversibly when heated.

The advantage of thermoplastics is that they are low-cost materials, can easily be shaped into various products, such as bottles, jugs and films, have some functional advantages, such as thermosealability and microwavability, and are mostly recyclable. Thermosets are mainly used for kitchen equipment and cookware, because of the demand for heat- and corrosion-resistant properties, which are needed to withstand exposure to acids in food and to cleaning materials. They are also used for their flame resistance around machined parts (in steamers, fryers, meat cutters and other kitchen equipment).

Plastics that are most often used as food packaging materials include polyolefines, polyesteres, polyvinyl chloride, polyvinylidene chloride, polystyrene, polyamide, various resins and so on. For example, the world plastic demand in 2011 was dominated by polyethylene, including low density polyethylene (PE-LD), linear low density polyethylene (PE-LLD, 17%) and high density polyethylene (PE-HD, 12%), polypropylene (PP, 19%), polyvinyl chloride (PVC, 11%), polystyrene solid (PS) and expandable (PS-E, 7.5%), polyethylene terephthalate (PET, 6.5%), polyurethane (PUR, 7%) and other plastic types (20%). An overview of the main types of polymeric packaging materials is given in Table 12.73.

Mass transfer, which is commonly referred to as **migration**, is a typical interaction of food with packaging made from plastics. Low molecular weight substances are transferred to foods, especially residual monomers and several thousand different types of additives (antioxidants, plasticisers, thermal stabilisers, lubricants, condensation components, slip additives, anti-static and anti-blocking agents). Characteristically, the packaging material is not completely destroyed, but retains technologically important properties, while only certain components diffuse into the food. Migration is always a two-way process, as some food constituents migrate into food packaging materials and sometimes significantly affect the rate of transfer of contaminants into the food. In terms of food quality, it is of course desirable to limit the transfer of substances in both directions. From the hygienic and toxicological point of view, the level of contaminants migrating into the food is particularly significant. In principle, two basic types of migration are distinguished:

- **overall** or **global** migration
- **specific** migration.

The overall (global) migration refers to the transfer of all, in many cases unknown, components from the packaging to the food. Table 12.74 shows the typical values for the overall migration of plastic packaging materials used for food packaging. Specific migration is the transition of one or more substances, which are particularly important from the hygienic and toxicological point of view. Because of the enormous complexity and number of potential contaminants and the extent of the use of polymeric packaging materials, specific migration is one of the most significant problems of food contamination from packaging.

Several thousand substances based on polymers are used in the production of packaging materials. Products resulting from chemical changes of these additives arising during the processing

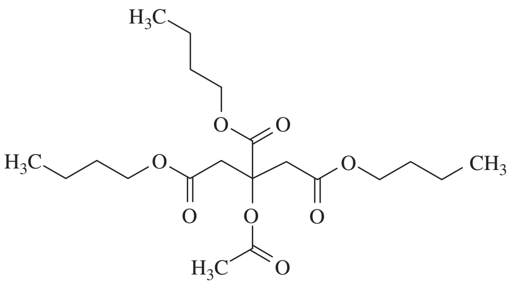
Table 12.73 Basic types of polymeric packaging materials.

Group of materials	Main types	Group of materials	Main types
Polyolefins	Polyethylene (PE-HD, PE-LD, PE-LLD) Polypropylene (PP)	Vinyl polymers	Polyvinyl chloride (PVC) Polystyrene (PS)
Nitrogen polymers	Polyamide (PA) Polyurethane (PUR)		Polyvinyl acetate (PVAc) Polyvinyl alcohol (PVOH)
Thermosets	Phenol-formaldehyde resins Amino-formaldehyde resins Epoxy resins	Polyesters	Polyethylene terephthalate (PET) Polycarbonate (PC) Thermoset polyesters

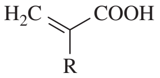
Table 12.74 Typical values of overall migration for plastic foils for food packaging.

	Migration (mg/dm^2)	
Polymer	**Aqueous simulants**	**Olive oil**
Low density polyethylene (PE-LD)	0.1–1.5	4–20
Linear low density polyethylene (PE-LLD)	0.1–1.0	1.5–5.5
Polyethylene terephthalate (PET)	<0.2	0.3–6.9
Polypropylene (PP)	0.1–1.5	0.5–5.0
Polystyrene (PS)	0.2–5	1.2–26
Polyamides (PA)	0.0–15	1.0–6,0
Polyvinyl chloride (PVC)	0.5–3	3.0–100

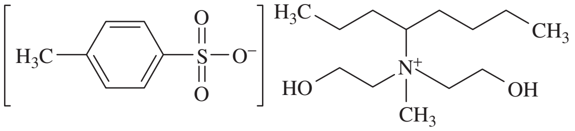
and use of packaging materials may also be released to food. An example is non-8-enal, an important contributor to plastic off-odor in polyethylene packaging, formed as a secondary product of oxidation of dodec-1-ene, which is one of the most abundant components of PE-HD volatiles. In practice, groups of structurally related compounds, such as phenols, primary and secondary amines and aromatic substances are often followed. The most important potential food contaminants are listed in Table 12.75, but this list is far from exhaustive. It does, however, illustrate the type of contaminants and the complexity of the potential contamination of foods from plastic packaging materials. Structures of the most important compounds are given in formulae **12-238** to **12-260**.



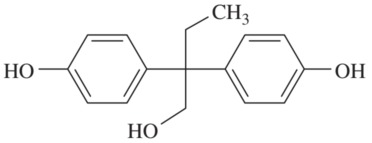
12-238, acetyl tributyl citrate



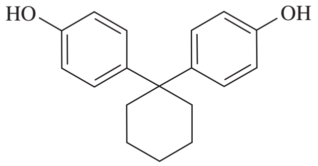
12-239, acrylic acid, R = H
methacrylic acid, R = CH_3



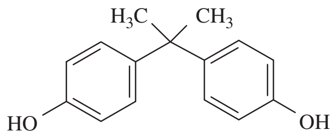
12-240, *N*,*N*-bis(2-hydroxyethyl)-*N*-(4-octyl)-*N*-methylammonium-*p*-toluenesulfonate



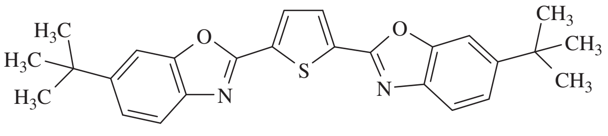
12-241, 2,2-bis(4-hydroxyphenyl)butan-1-ol



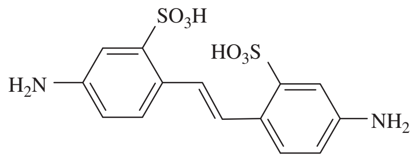
12-242, 1,1-bis(4-hydroxyphenyl)cyclohexane



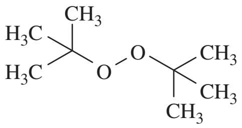
12-243, bisphenol A



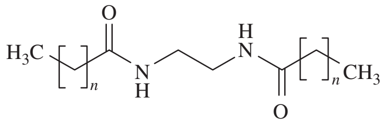
12-244, 2,5-bis(5-*tert*-butylbenzoxazol-2-yl)thiophene



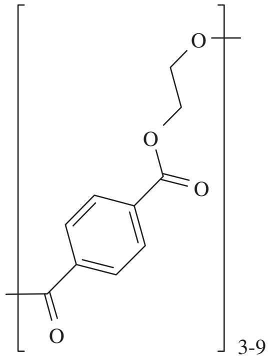
12-245, 4,4′-diamino-2,2′-stilbenedisulfonic acid



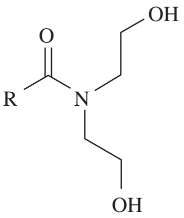
12-246, di-*tert*-butylperoxide



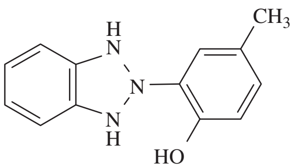
12-247, *N*,*N*¢-dipalmitoylethane1,2-diamine, *n* = 14
N,*N*¢-distearoylethane-1,2-diamine, *n* = 16



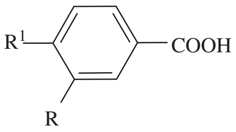
12-248, ethyleneterephthalate oligomers



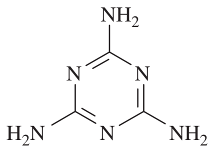
12-249, fatty acid diethanolamides



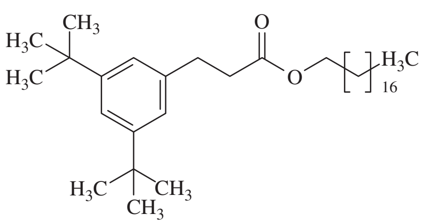
12-250, 2-(2-hydroxy-5-methylphenyl)benzotriazole



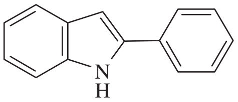
12-251, isophthalic acid, R = COOH, R^1 = H
terephthalic acid, R = H, R^1 = COOH



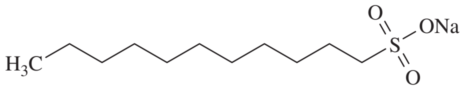
12-252, melamine



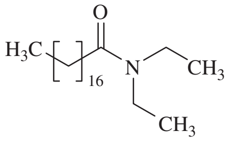
12-253, octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate



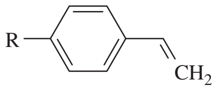
12-254, 2-phenylindole



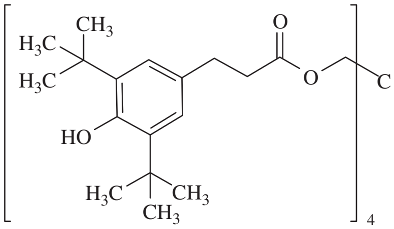
12-255, sodium 1-undecanesulfonate



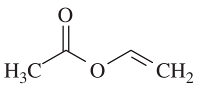
12-256, stearic acid *N*,*N*-diethylamide



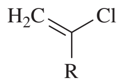
12-257, styrene, R=H
4-methylstyrene, R = CH_3



12-258, tetrakis[methylene-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) propionyl]methane



12-259, vinyl acetate



12-260, vinyl chloride, R = H
vinylidene chloride, R = Cl

Table 12.75 Classification of contaminants from packaging materials.

Groups of contaminants	Compounds and examples of their occurrence[a]
Residues of monomers	Adipic acid dimethyl adipate (polyesters), acrylamide (acrylonitrile co-polymers), acrylonitrile (acrylamide co-polymers), acrylic acid (acrylic polymers), buta-1,3-diene (PS co-polymers, elastomers), butan-1,4-diol (polyesters), 2,2-bis(4-hydroxyphenyl)butan-1-ol (polyesters), 1,1-bis(4-hydroxyphenyl)cyclohexane (polyesters), 4,4′-(propane-2,2-diyl)-diphenol known as bisphenol A (polyesters), ethyleneglycol (polyestere), formaldehyde (phenol-formaldehyde resins), isophthalic acid (PET), caprolactame and C_6-C_{12} aminocarboxylic acids and their lactames (polyamides), melamine (amino-formaldehyde resins), methacrylic acid and methyl methacrylate (acrylic polymers), methylstyrene (PS and co-polymers), propyleneglycol (polyesters), sebacic acid and dimethyl sebacate (polyesters), styrene (PS and co-polymers), terephthalic acid and dimethyl terephthalate (PET), vinyl acetate (vinyl acetate co-polymers), vinyl chloride (PVC and co-polymers), vinylidenechloride (PVdC and co-polymers)
Residues of additives	
Initiators	Di-*tert*-butylperoxide, dibenzoylperoxide
Regulators	Dodecylmercaptan
Catalysts	Oxygen compounds of Al, Ti, Ca, Mg, Si, Cr, Sb, Mn, Li, Zn, Co and others
Emulsifiers	Sodium 1-alkylsulfonates (C_{12}–C_{18})
Protective colloids	Polyvinyl alcohol, lecithin, gelatin, carboxymethylcellulose
Solvents	Acetone, butyl acetate, dichloromethane, ethanol, ethyl acetate, hexane, pentane, toluene
Hardeners	Formaldehyde, glutaraldehyde, glyoxal and others
Stabilisers and antioxidants	2- and 3-*tert*-Butyl-4-hydroxyanisole (BHA), 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT), 2-ethyl-dioctyl-tin-di-2-ethylhexyl-monooctyltintrithioglycolate (Irgastab 17 MOK), 2-phenylindole, octadecyl 3-(3,5-di-*terc*-butyl-4-hydroxyphenyl)-propionate(trade name Irganox 1076), tetrakis[methylene-3-(3,5-di-*terc*-butyl-4-hydroxyphenyl)propionyl]-methane (trade name Irganox 1010)
Plasticisers	Acetyl tributyl citrate, butylesters of soybean oil fatty acids, butyl stearate, dibutyl adipate, dibutyl sebacate, dioctyl adipate, dioctyl sebacate, epoxidised soybean oil, phthalates, glycerol, isobutyl stearate and others
Lubricants	Stearic acid diethylamide, *N*,*N*′-dipalmitoylethane1,2-diamine, *N*,*N*′-distaroylethane1,2-diamine, glycerol, stearic and palmitic acids, silicone oils, Ca, Mg, Al and Zn stearates
Antistatic agents	Fatty acid diethanolamides, *N*,*N*-bis(2-hydroxyethyl)-*N*-4-octyl-*N*-methyl-ammonium-*p*-toluenesulfonate
UV absorbers	2,4-Dihydroxybenzophenone, 2-(2-hydroxy-5-methylphenyl)benzotriazole
Optical lighteners	2,5-Bis(5-*tert*-butylbenzoxazol-2-yl)thiophene, 4,4′-diamino-2,2′-stilbenedisulfonic acid
Fillers	Silica, kaolin, graphite
Blowing agents	Isopentane or pentane, petroleum ether
Degradation products (of polymers and additives)	Acetaldehyde (PET), oligomers (PET), bisphenol A (polyesters), hydrochloric acid (PVC), dibutyl adipate (PVC), nitrosamines (elastomers, rubber)

[a] PS = polystyrene, PET = polyethylene terephthalate, PVC = polyvinyl chloride, PVdC = polyvinylidene chloride.

Table 12.76 Physical and chemical properties of phthalates.

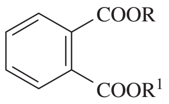
Phthalate	M_r	Boiling point (°C)	Melting point (°C)	Solubility in water (mg/l at 25 °C)	Vapour pressure (mPa) (25 °C)	pK_{OW}
Dimethyl phthalate	194.2	284	2	4000	220	1.53
Diethyl phthalate	222.2	294	−41	1080	220	2.35
Dibutyl phthalate	278.4	340	−35	11.2	1.87	4.57
Benzylbutyl phthalate	312.4	370	−35	2.69	1.15	4.91
Bis(2-ethylhexyl) phthalate	390.6	384	−50	0.3	0.86	5.11
Dioctyl phthalate	390.6	220 (at 670 Pa)	−25	0	-	-

12.8.1.5.1 Phthalates

Phthalates (esters of benzene 1,2-dicarboxylic acid, known as phthalic acid) belong to the group of virtually ubiquitous organic environmental contaminants. The reason for their abundant spread to all components of the environment is their suitable physico-chemical properties, which is why phthalates are used as plasticisers, substances improving the mechanical properties of plastics (mainly PVC), their flexibility, transparency, durability and longevity. Phthalates therefore occur in a wide variety of products ranging from common household products, such as floor coverings, curtains in bathrooms, rubber gloves and children's toys through to food packaging to specialised products used, for example, in health care and in bags for storage of blood and blood plasma, syringes and dialysis units. Plastic materials treated with phthalates may contain up to 40% plasticiser, which is not chemically bound to the polymer and may thus be released into foods that are in contact with plastics.

Structure and nomenclature

The U.S. EPA included six phthalic acid esters (**12-261**) on the list of priority environmental contaminants. Of these, the most widespread are dibutyl phthalate and bis(2-ethylhexyl)phthalate.



12-261, phthalates

dimethyl phthalate, R = R^1 = CH_3
diethyl phthalate, R = R^1 = CH_2CH_3
dibutyl phthalate, R = R^1 =$[CH_2]_3CH_3$
benzylbutyl phthalate, R = $CH_2C_6H_5$, R^1 = $[CH_2]_3CH_3$
bis(2-ethylhexyl) phthalate, R = R^1 = $CH_2CH(CH_2CH_3)$ $[CH_2]_3CH_3$
dioctyl phthalate, R = R^1 = $[CH_2]_7CH_3$

Properties

The phthalates molecule is a rigid planar aromatic ring with two flexible, generally non-linear, different or identical aliphatic side chains. Phthalates are odourless, relatively highly lipophilic liquids, with low vapour pressure and high boiling points (Table 12.76). Their lipophilicity (partition coefficient octanol–water, K_{OW}) increases with increased molecular weight. Like other esters, phthalates are easily hydrolysed in acidic and basic solutions (Figure 12.69). Owing to their low solubility in water, however, this reaction is very slow. Esters with branched bulky substituents, such as bis(2-ethylhexyl) phthalate, are more resistant to hydrolysis than the straight chain lower esters (e.g. dibutyl phthalate).

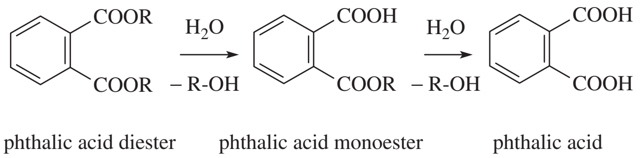


Figure 12.69 Hydrolysis of phthalates.

Occurrence and main sources

The main and probably the only source of phthalates is human activity. The release of phthalates into the environment occurs not only during their manufacture and production of materials containing phthalates, but also during use and subsequent disposal of materials containing phthalates. The atmosphere, waste water, soil and also foods may be contaminated.

Evaporation of phthalates in the atmosphere is negligible due to their low vapour pressure. Higher concentrations can occur near plants producing phthalates, or in enclosed areas (e.g. in cars and rooms with new flooring). Contamination in industrial centres or enclosed areas can reach values of tens of mg/m^3. In the air over the oceans, concentrations of phthalates are at the level of ng/m^3. As with other persistent contaminants, phthalates may be in the form of vapour (esters with lower relative molecular weights), aerosols or bound to dust particles. Phthalates can be transported over long distances in this form, especially bis(2-ethylhexyl) phthalate, or washed out by rain and snow precipitation.

Contamination of surface and groundwater can be caused by wastewater, solid waste and indirectly by rainfall. Amounts of phthalates vary by location and differ within one tenths to thousands

of μg/l, but in sediments, concentrations of hydrophobic phthalates can reach hundreds of mg/kg.

The main sources of soil contamination are industrial and municipal waste, agrochemicals and emissions. In the soil, phthalates tend to adsorb to organic matter, where they accumulate. The soil adsorption coefficient K_{OC} increases with the relative molecular weight of phthalates and their decreasing solubility in water, so that bis(2-ethylhexyl) phthalate is strongly sorbed and essentially immobile, while dimethyl phthalate is very mobile. Dimethyl and diethyl phthalates can easily leach into groundwater from soils and may be partially evaporated.

The migration of phthalates from packaging materials into food is generally influenced by many factors, such as:

- type of polymeric packaging material
- type of food (the amount of fat is important)
- temperature
- length of contact with foods.

The most important source of phthalates is food intake, but data on contamination of food are very limited. Generally, food contamination by phthalates may be in the range of hundredths up to units of mg/kg, with occasional extreme values primarily in fatty foods, such as cheeses, cakes and sandwiches that may readily contain phthalates in amount of tenths of mg/kg food. The occurrence of phthalates in food may be the result of contamination of raw materials, intermediates or finished products. In Europe, the estimated average maximum exposure to bis(2-ethylhexyl) phthalate from packaging is estimated at 0.02 mg per person per day, and exposure to all phthalates (expressed as dimethyl phthalate) is estimated to be 4.37 mg per person per day. Daily intake of bis(2-ethylhexyl) phthalate from food in the Netherlands is estimated at 0.5–0.8 mg, 2 mg in Japan and 0.25 mg in the United States (the total intake from food, water and air is about 0.27 mg).

Another source with respect to a specific population is blood transfusions, when the patient can obtain up to 300 mg in a single transfusion, and during dialysis of blood when the patient may receive 40 mg of bis (2-ethylhexyl) phthalate per day. In industrial plants with a maximum permissible concentration of bis(2-ethylhexyl) phthalate 5 mg/m, the daily intake of this phthalate by workers reaches 20 mg.

Degradation and metabolism

Hydrolysis and photodegradation of phthalic acid esters in the abiotic components of the environment is extremely slow, and higher esters, such as bis(2-ethylhexyl) phthalate and dioctyl phthalate, are virtually stable. The major route of elimination of phthalates from the environment is their biodegradation. Almost all organisms have the biochemical means (non-specific esterases) to be able to catalyse the hydrolysis of phthalates. Microbial degradation of phthalates occurs mainly under aerobic conditions in soil and by aquatic bacteria and fungi. The degradation process begins through hydrolysis to phthalic acid monoesters and subsequently to free phthalic acid. Degradation of phthalic acid via pyruvate and succinate produces carbon dioxide and water.

Immediately after entering the human organism via the lungs (by respiration) and intestines (via food), phthalates get into the blood and advance to the liver, kidney and testes. To a limited extent they are stored in the fat tissue. Metabolism of bis(2-ethylhexyl) phthalate begins by hydrolysis in the gastrointestinal tract to 2-ethylhexyl phthalate and 2-ethylhexan-1-ol. In the next metabolic step a small proportion of 2-ethylhexyl phthalate may be hydrolysed to phthalic acid and 2-ethylhexan-1-ol, but the major proportion of the monoester is oxidised in the aliphatic side chain to yield various metabolites (about 30 metabolites have been identified). In most mammals, 2-ethylhexyl phthalate and its oxidised derivatives react with D-glucuronic acid to form conjugates, which are excreted from the body. Metabolism of phthalates with shorter side chains is similar. Despite the relatively rapid metabolism and excretion of phthalates (60–90% is excreted in urine and faeces within 24 h), a certain proportion of lipophilic phthalates are accumulated in the body, because the rate of their intake exceeds the rate of metabolic conversion.

12.8.2 Health and toxicological assessment

Packaging materials are not considered toxic, but may contain toxic degradation products and many additives and plastics may occur as non-bound residual monomers, which have toxic properties. There is a risk with any packaging material that its components may be transferred in some way to the food. Fortunately, the level of contaminant transfer is extremely low in most cases, however, there are instances where a real hazard exists and must be controlled. No official internationally agreed guidelines exist, but in the EU there is a general requirement that food packaging components must not be transferred into food during its normal shelf life, pose a health risk, or adversely affect the quality, flavour, texture or appearance of the food. The major area of concern is the transfer of plastic material monomers and additives, especially plasticisers. The residual monomer content depends on polymer type and polymerisation technique. The contents of the monomers in various polymers vary from very low levels (mg/kg levels) up to 40 000 mg/kg. The amounts of additives used are also highly variable. Polyvinyl chloride (PVC), for example, requires the highest levels of additives, and accounts for 73% of the world's production of additives by volume, while polypropylene and polyethylene account for 10% and styrene for 5%. The toxicological aspects of these contaminants are briefly described in the following sections.

12.8.2.1 Plastic material monomers

In 1974, the monomer of PVC, vinyl chloride (chloroethene), was first reported to cause angiosarcoma (a form of tissue cancer that arises in the lining of blood vessels) of the liver both in humans and in animals. Additional research has demonstrated its

carcinogenicity to other organs. The target organs include the liver, brain, lung and probably the lymphohaematic system. According to the IARC, vinyl chloride is a Group 1 agent carcinogenic to humans. Dietary exposure to vinyl chloride is controlled by limiting either its content in food-contact material (typically less than 1 mg/kg) or migration to foods at the lowest technically achievable level (typically less than 0.01 mg/kg). Vinylidene chloride has some detrimental effects in mice and rats, but no carcinogenic effects have been seen. It is probably not carcinogenic to humans (a Group 3 agent).

Acrylonitrile, which is mutagenic after metabolic activation in the liver, is considered more toxic than chlorinated monomers and is possibly carcinogenic to animals and humans as a Group 2B agent. It is metabolised to cyanides, which are subsequently transformed into thiocyanates and excreted in the urine.

Reports of organ toxicity upon chronic exposure to styrene are rare; however, since the main intermediate in styrene metabolism is an epoxide (styrene-7,8-oxide), hepatotoxicity due to covalent binding at the site of formation appears to be a possibility. Both of these substances, styrene (a Group 3 agent) and its oxide (recently upgraded to a Group 2A as probably carcinogenic to humans) have been shown to produce chromosomal aberrations under certain conditions.

12.8.2.2 Plastic material additives

Among the additives used to modify the properties of polymeric packaging materials, plasticisers have raised much concern from the hygienic point of view. Butyl stearate, acetyltributyl citrate, alkyl sebacates and adipates are important because they are types of plasticisers that typically have low toxicities. Materials such as epoxidised soybean oil are widely used in polyvinyl chloride, polyvinylidene chloride and polystyrene as thermal stabilisers and lubricants at a level of 0.1–27%. Toxicity of epoxidised soybean oil is affected by the presence of oxirane, also known as ethylene oxide, which was upgraded to Group 1 as a carcinogenic agent to humans, based on mechanistic and other relevant data.

However, restrictions have been brought in on the use of phthalate plasticisers due to their potential carcinogenic effects. The risks from the presence of phthalates have been broadly discussed for decades, but only in the 1980s did suspicions over the possible carcinogenic effects of bis(2-ethylhexyl) phthalate, and in the 1990s on the weak oestrogenic activities (possibly related to increased incidence of testicular cancer or breast cancer) of dibutyl phthalate and benzylbutyl phthalate, result in interest in these compounds. The acute toxicity of phthalates is low and is manifested by gastrointestinal irritation, nausea, sleepiness, low blood pressure, dizziness, hallucinations, blurred vision and tearing. Inhalation of vapours leads to coughing and irritation of the throat and oesophagus. In terms of subacute and chronic effects, phthalates with branched side chains are dangerous, such as bis(2-ethylhexyl) phthalate. The primary target organs are the liver, which manifests by liver enlargement, increased pigmentation, fat sediments, increasing number of hepatic peroxisomes and mitochondria. The kidneys and other organs may also be affected. Chronic intake of phthalates, particularly of bis(2-ethylhexyl) phthalate, may likewise have teratogenic and carcinogenic effects (liver cancer) and might affect the reproductive ability of the body (weight reduction of testes, ovaries and sperm count). With regard to possible carcinogenic effects, bis(2-ethylhexyl) phthalate is classified as a possibly carcinogenic agent to humans (Group 2B) and butybenzyl phthalate as an agent that is not classifiable as to its carcinogenicity to humans (Group 3).

Bisphenol A occurs in polycarbonate plastics that have many applications, including use in some food and drink packagings, such as water and infant bottles, compact discs, impact-resistant safety equipment and medical devices, as well as in epoxy resins used as lacquers (such as the resin with the trade name Araldite). It is an endocrine disruptor, which has a high oestrogenic potential, and can mimic the natural hormone 3,17β-oestradiol, which may lead to negative health effects. Some studies have linked prenatal exposure to bisphenol A with later physical and neurological difficulties. Other studies have also presented a hypothesis that the current level of human exposure to growth hormones that fatten livestock, to pharmaceuticals that induce weight gain and, in addition, to bisphenol A can damage many of the body's natural weight-control mechanisms. Furthermore, it is posited that these effects, together with a wide range of additional, possibly synergistic factors, may play a significant role in the worldwide obesity epidemic. In 2010, Canada became the first country to declare bisphenol A a toxic substance. The European Union, Canada and recently the United States have banned the use of bisphenol A in baby bottles.

12.8.3 Mitigation

Requirements for the safety of food packaging materials and articles intended to come into contact with food are regulated by the Regulation (EC) No. 1935/2004 of the European Parliament and of the Council on materials and articles intended to come into contact with food. In addition to this document there are also requirements directly applicable in EC regulations, which are in particular: Commission regulation (EC) No. 1895/2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food, Commission regulation (EC) No. 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food, Commission regulation (EC) No. 372/2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods and Commission regulation (EC) No. 282/2008 on recycled plastic materials and articles intended to come into contact with foods.

The legislation is based on three principles: positive list of substances, which may be used for the production of objects coming into contact with food; determination of specific migration limits for substances with proven adverse health effects; and compliance with the principles of good manufacturing practice for manufacturers of these items. For example, plastic materials and objects shall not transfer their constituents to foods in quantities exceeding 60 mg/kg of food, which is called the **Overall Migration Limit** (OML). In some cases, this limit may be expressed rather as 10 mg/dm^2 of surface of the material or object. To

determine whether a particular plastic formulation meets these criteria, legislative regulations describe in detail the terms and procedures of migration tests that are commonly performed not on individual foods, but on standardised food stimulants, which represent different conditions for leaching of undesirable substances. These are: distilled water, 3% aqueous solution of acetic acid, 10% aqueous solution of ethanol (or greater, if the alcoholic beverage in question has higher alcohol content) and olive oil. The regulations also specify which simulants should be used for each category of food. In general, there are no simulants listed for dried foods, which can be considered not to take up plastic constituents from contact materials. Very strict criteria in terms of the migration of harmful substances are also set for paper and cardboard, which are the traditional packaging materials coming into contact with food.

For substances with proven toxic effects, **Specific Migration Limits** (SMLs) have been declared in food simulants or the maximum amounts in a given material for unstable compounds are stated. An example can be the SML for melamine (2,4,6-triamino-1,3,5-triazine), which is set at 30 mg/kg. On the other hand, acrylamide cannot be detected at all in the migration tests (its detection limit is 0.01 mg/kg), although it is known as a common process contaminant. The SML value for dibutyl phthalate is 0.3 mg/kg and for bis(2-ethylhexyl) phthalate 1.5 mg/kg, but plasticised plastics should not be used for fatty foods. In sealing lids for jars intended for infant and baby food, the migration of epoxidised soybean oil is often controlled (SML = 30 mg/kg).

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